UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE – UFCSPA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

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EFEITOS DO TREINAMENTO DE FORÇA ISOLADO E COMBINADO AO TREINAMENTO AERÓBICO SOBRE A FUNÇÃO HEMODINÂMICA, DEPOSIÇÃO DE COLÁGENO E MARCADORES INFLAMATÓRIOS EM RATOS COM INSUFICIÊNCIA CARDÍACA

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Tese submetida ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Ciências da Saúde de Porto Alegre como requisito para a obtenção do grau de Doutor

Orientador: Prof. Dr. Pedro Dal Lago

Porto Alegre
2016

Catalogação na Publicação

Pereira Alves, Jadson
Efeitos do treinamento de força isolado e combinado ao treinamento aeróbico sobre a função hemodinâmica, deposição de colágeno e marcadores inflamatórios em ratos com insuficiência cardíaca / Jadson Pereira Alves. -- 2016.
167 p. : il., graf., tab. ; 30 cm.

Tese (doutorado) -- Universidade Federal de Ciências da Saúde de Porto Alegre, Programa de Pós-Graduação em Ciências da Saúde, 2016.

Orientador(a): Pedro Dal Lago.

1. Exercício físico. 2. Treinamento de força. 3. Infarto do miocárdio . 4. Insuficiência cardíaca. 5. Remodelamento cardíaco. I. Título.

Sistema de Geração de Ficha Catalográfica da UFCSPA com os dados fornecidos pelo(a) autor(a).

AGRADECIMENTOS

Agradeço aos meus pais pelo amor despendido durante a minha infância e adolescência. Formavam um casal muito feliz, mas por obra do destino tiveram que partir. Muito obrigado pelo aprendizado.

Aos meus irmãos e irmãs que mesmo distantes da minha vida conseguiram contribuir com a minha formação pessoal, mas em especial agradeço a minha querida irmã Liana e ao meu irmão Jaider.

Agradeço a minha namorada Fabiana Sá Rebello pelo amor, sensibilidade, paciência e apoio nesse momento de aprendizado pessoal e acadêmico. São muitos anos de parceria que deixaram esse período um pouco mais leve.

Aos pais da minha namorada, Lucia Sá Rebello e Paulo Seibert Rebello, pelo carinho e companheirismo.

Ao meu orientador Prof. Dr. Pedro Dal Lago que acreditou em uma linha de trabalho que muitos duvidaram que fosse possível executar. Agradeço pelos ensinamentos, confiança, contribuições que foram essenciais para a minha formação acadêmica e de vida. Muito obrigado por tudo!

Agradeço aos meus colegas e professores da área das artes marciais que de alguma forma me educaram e ensinaram a respeitar um sistema hierárquico.

Especialmente eu agradeço ao meu grande amigo e irmão Prof. Dr. Ramiro Barcos Nunes que sempre me ajudou e proporcionou o melhor ambiente de trabalho que eu poderia ter, foram muitas convivências e trocas de experiência que hoje me tornam um ser humano melhor. Não irei esquecer jamais da frase: “o mundo é de quem faz, não é de quem pode e nem de quem quer”.

Ao amigo Me. Giuseppe Potrick Stefani que vivenciou todo o início desse protocolo ousado e que me acompanhava de segunda a segunda nas atividades do laboratório, sempre prestativo e muito empolgado com cada conhecimento adquirido.

Ao meu amigo Prof. Dr. Rodrigo Boemo Jaenisch que salvou muitas cirurgias onde eu mesmo não acreditava que poderia ser possível continuar, sou grato pelos ensinamentos técnicos e trocas de informações diária.

Agradeço ao amigo Prof. Dr. Vítor Scotta Hentschke que também se desafiou no protocolo de treinamento de força para ratos e me ajudou a organizar de uma forma melhor todos os experimentos.

Agradeço à Daniele Ferreira que fez a iniciação científica nesse trabalho e foi essencial nos procedimentos experimentais, sempre atenta para que tudo desse certo.

Ao amigo e companheiro de longa jornada Prof. Dr. Thiago Gomes Heck pela grande contribuição na minha formação e por oportunizar momentos intensos de ciência.

Agradeço ao amigo Prof. Dr. Luís Fernando Deresz que me ensinou muito durante esse período de formação, foram muitos almoços com troca de informações essenciais para a minha vida.

Agradeço ao grande amigo Prof. Dr. Edson Quagliotto que sempre se mostrou disponível para me ajudar e, para mim, é um exemplo de humildade e perseverança.

Ao amigo e grande Prof. Dr. Anderson Zampier Ulbrich que é um exemplo de pessoa e me ensinou muito durante esses anos, pois a sua batalha na vida me inspirou a seguir adiante.

À colega Giovanna Barcelos que participava dos experimentos e sempre se mostrou flexível na utilização dos aparatos laboratoriais.

Agradeço a todos os colegas de laboratório que de certa forma contribuíram para a minha formação acadêmica e me proporcionaram grandes momentos de aprendizado. Destaco entre eles o Prof. Dr. Marlus Karsten, Prof. Me. Douglas Rossato, Me. Ananda Rucatti, Me. Deise Pacheco, Prof. Me. Daniela Hass, Me. Jéssica Lima, Me. Ana Paula Salazar, Me. Ana Amélia Duarte, Júlia Perini e Juliano Lazzari.

Em especial agradeço aos meus queridos amigos de longa data, Amilcar Ricaldes Palmeira (Mika), Robson Roberto Rodrigues (Pinto), Alfredo Bermudez (Psico), Raquel Quartiero (Canjica), Neivaldo Pinheiro (Nei), Marcelo Brandão (Peninha), André Gustavo de Azevedo Barreto (Tatuíra), Sergio Liuche (Magoo) e Luciano Brasil (Luc).

À Prof. Dra. Marcia Regina Vitollo, pela sua amizade de longa data e conselhos que sempre me ajudaram tanto na formação acadêmica como na vida.

À secretaria da pós-graduação, especialmente à Luciane Spencer, Maristela Pasin, Isadora Farias, Daniela Dalpiaz e Cristiane Mandadori por todo suporte, eficiência e dedicação.

Ao laboratório de patologia da UFCSPA, especialmente à Teresinha Stein, Rosalva Theresa Meurer e à Professora Marilda da Cruz Fernandes pelo suporte técnico.

À Prof. Dra. Claudia Ramos Rhoden e aos seus alunos do laboratório de Poluição Atmosférica e Estresse Oxidativo pelo apoio prestado e muitas risadas.

À Ignez do biotério pelos cuidados com os animais e pela parceria no dia a dia.

Às técnicas Carmem Rocha e Monice Santos pelo apoio e disposição em atender as demandas do laboratório.

Ao Mário Serapião, pelo enorme apoio prestado durante a minha iniciação no laboratório, uma pessoa de grande coração e ótimo assador de churrasco, mas que infelizmente não se encontra mais entre nós.

“O que prevemos raramente ocorre;
o que menos esperamos geralmente acontece.”
Benjamin Disraeli

Resumo

A insuficiência cardíaca (IC) é associada a uma desorganização da arquitetura muscular e do metabolismo, contribuindo diretamente para intolerância ao exercício, fragilidade e mortalidade. A fraqueza muscular no cenário da IC tem sido associada com aumento da mortalidade, ao passo que elevados ganhos na força muscular podem prolongar o tempo de sobrevida desses pacientes. Dessa forma, o treinamento de força (TF) tem sido associado com positivas respostas frente as doenças cardiovasculares (DCV) e, quando ocorre associação com o treinamento aeróbico (TA), os efeitos parecem ser mais pronunciados. Os potenciais benefícios do TF de alta intensidade na IC são poucos difundidos na literatura e quando associado ao TA tornam-se mais escassos. Além disso, a quantificação da dose resposta do exercício físico, especialmente do TF, não está bem elucidada no campo das DCV. Adicionalmente, é bem definido que o TF é uma modalidade efetiva para estimular a hipertrofia muscular e melhorar a força, mas a manipulação das variáveis de treinamento como frequência, intensidade e volume são raramente comentadas no complexo cenário da IC. O modelo experimental de IC induzido pelo método de infarto do miocárdio (IM) é amplamente utilizado para a melhor compreensão dos potenciais efeitos das mais variadas estratégias terapêuticas. O modelo de agachamento para animais de pequeno porte tem demonstrado adaptações significativas tanto no sistema cardiovascular como no sistema muscular e algumas investigações já demonstraram que esse modelo pode servir para responder alguns questionamentos de característica translacional. Portanto os objetivos dessa tese são: 1) avaliar os efeitos do TF de alta intensidade e TA isolados ou combinados sobre os parâmetros hemodinâmicos, deposição de colágeno e marcadores inflamatórios (IL-10 e TNF-α) em ratos com IC induzida pelo método de IM; 2) avaliar os efeitos do TF de alta intensidade isolado ou combinado com TA sobre a magnitude do ganho de força (teste de 1RM) e capacidade funcional (velocidade, tempo e distância percorrida na esteira através do teste de tolerância máximo ao exercício); 3) Avaliar a influência do volume de séries e repetições do TF de alta intensidade isolado ou combinado ao TA sobre os ganhos de força e capacidade funcional de animais saudáveis e animais com IC induzida por IM. Através dos estudos realizados nessa presente tese conclui-se que: 1) o TF de alta

intensidade isolado ou combinado com o TA foi capaz de aumentar o ganho de força muscular, e a maior concentração desse ganho de força está entre os testes que ocorreram nas duas primeiras semanas de treinamento (1º 1RM *vs* 2º 1RM); 2). Os protocolos de exercício físico isolados ou combinados com TA foram capazes de atenuar a disfunção ventricular esquerda dos animais com IC; 3) os animais submetidos aos protocolos de TA isolado e ao treinamento combinado apresentaram melhora na capacidade funcional através do teste de tolerância máxima ao exercício físico (velocidade, tempo e distância); 4) a deposição de colágeno no coração dos animais com IC foi atenuada em todos os protocolos de treinamento físico; 5) os animais com IC treinados não apresentaram redução na capacidade de ganhar força muscular e capacidade funcional quando comparados com animais controle saudáveis treinados; 6) o aumento do volume de séries e repetições apresentou associação com a atenuação da pressão diastólica final do ventrículo esquerdo (PDFVE) e o volume de repetições também foi associado com atenuação do volume de colágeno intersticial do coração de animais com IC.

Os resultados combinados dos dois estudos indicam que o TF de alta intensidade isolado ou combinado com TA pode ser uma boa estratégia para aumentar a força dos animais e consequentemente atenuar a disfunção ventricular esquerda e atenuar o remodelamento cardíaco adverso. Além disso, o equilíbrio entre dose e resposta do exercício físico, especialmente TF, pode ser melhor ajustado por meio da manipulação do volume e intensidade nos modelos experimentais de IM visando respostas mais consistentes na área dos marcadores inflamatórios. Adicionalmente, os animais com IC apresentam ganhos similares comparados aos animais controle saudáveis tanto na capacidade de gerar força muscular como na capacidade funcional, tornando esse um bom modelo experimental para futuras investigações no campo das adaptações teciduais em animais com IC.

Abstract

Heart failure (HF) is associated with a derangement of muscle architecture and metabolism, directly contributing to exercise intolerance, weakness and mortality. Muscle weakness in the context of HF has been associated with increased mortality, while the gain in muscle strength can increase the survival time of these patients. Thus, resistance training (RT) has been associated with positive responses in the field of cardiovascular disease, and when combined with aerobic training (AT) the effects appear to be even more pronounced. The potential benefits of high-intensity RT in HF are few disseminated in literature and when combined with AT becomes scarcer. Furthermore, the quantification of the dose-response of physical exercise, especially RT, is not well elucidated in the field of cardiovascular diseases. Additionally, it is well established that RT is an effective modality to stimulate muscle hypertrophy and improve strength, but the manipulation of training variables such as frequency, intensity and volume are seldom discussed in the complex scenario of HF. The experimental model of heart failure induced by the method of acute myocardial infarction (AMI) is widely used for better understanding of the potential effects of therapeutic strategies. The squat model for small animals has demonstrated significant adaptations in both the cardiovascular system and the muscular system and some investigations have shown that this model can serve to answer some questionings translational feature. Therefore, the thesis objectives are: 1) to evaluate the effects of high-intensity RT and AT alone or in combination on hemodynamics, collagen deposition and inflammatory markers (IL-10 and TNF-α) in rats with heart failure induced by method AMI; 2) evaluate the effects of high-intensity RT alone or in combination with AT on the magnitude of the gain strength (1RM test) and functional capacity (speed, time and distance traveled in the maximum tolerance test to exercise); 3) evaluate the influence of the volume of sets and reps of high-intensity RT alone or combined with AT on strength gains and functional capacity of healthy animals and animals with heart failure induced by myocardial infarction. Through the studies in this present thesis is concluded that: 1) high-intensity RT isolated or combined with AT was able to increase the gains in muscle strength and the greatest concentration of strength gain is among the tests that have occurred in the first two weeks of training (1st 1RM *vs* 2nd 1RM); 2) the exercise protocols alone

or combined with AT were able to attenuate left ventricular dysfunction of the animals with HF; 3) The animals subjected to RT protocols isolated and combined training exhibited an improvement in functional capacity through the maximum tolerance test to exercise (speed, time and distance); 4) the collagen deposition in the heart of the animals with HF was attenuated in all physical training protocols; 5) the animals trained with HF did not show reduced ability to gain muscle strength and functional capacity when compared with healthy control animals trained; 6) the increased volume of sets and repetitions was associated with attenuation of left ventricular end-diastolic pressure (LVEDP) and the volume of repetitions was also associated with attenuation of interstitial collagen volume in the heart of animals with HF. The combined results of the two studies indicate that high-intensity RT alone or combined with AT may be, in part, a good strategy to increase the strength of the animals and thus attenuate left ventricular dysfunction and adverse cardiac remodeling. Moreover, the balance between dose-response to exercise, especially in the RT, it might be better adjusted by manipulating the volume and intensity in experimental models of AMI targeting more consistent responses in the area of inflammatory markers. Additionally, animals with HF presented similar gains compared to healthy control animals both in the capability to generate muscle strength and functional capacity, making this a good experimental model for future research in the field of tissue adaptations in animals with HF.

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Estudo 1

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Lista de abreviaturas do referencial teórico

AHA: *American Heart Assosiation*

DCV: Doenças cardiovasculares

- dP/dt_{max}: Derivada máxima negativa da pressão ventricular

+ dP/dt_{max}: Derivada máxima positiva da pressão ventricular

FEVE: Fração de ejeção do ventrículo esquerdo

IM: Infarto do miocárdio

IC: Insuficiência cardíaca

NYHA: *New York Heart Association*

PA: Pressão arterial

PAD: Pressão arterial diastólica

PAM: Pressão arterial média

PAS: Pressão arterial sistólica

PDFVE: Pressão diastólica final do ventrículo esquerdo

PDVE: Pressão diastólica do ventrículo esquerdo

PGC - 1α: *Peroxisome proliferator-activated receptor gamma coactivator 1-alpha*

PSVE: Pressão sistólica ventricular esquerda

RC: Reabilitação cardíaca

TAC: Treinamento aeróbico contínuo

TF: Treinamento de força

TNF-α: Fator de Necrose Tumoral Alfa

VE: Ventrículo esquerdo

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1. Introdução

A insuficiência cardíaca (IC) é uma síndrome clínica complexa que resulta em desordens funcionais e estruturais do músculo cardíaco, levando a sintomas como dispneia e fadiga, o que pode resultar em intolerância ao exercício nesses pacientes (YANCY *et al.*, 2013). Consequentemente a esse quadro, a IC apresenta alta mortalidade, frequente hospitalização e reduzida qualidade de vida exigindo um complexo regime terapêutico (HEART FAILURE SOCIETY OF *et al.*, 2010). O curso da doença leva a alterações periféricas na musculatura esquelética que estão intimamente relacionadas com a fraqueza muscular (MILLER *et al.*, 2009). Esse cenário proporciona limitações para a performance cardíaca e capacidade ao exercício físico desses pacientes (ZIZOLA & SCHULZE, 2013).

Atualmente, muitas investigações procuram esclarecer o complexo cenário da fraqueza muscular, pois o desenvolvimento da força muscular esquelética parece estar associado com a sobrevida de pacientes com IC (ANKER *et al.*, 1997; HULSMANN *et al.*, 2004; IZAWA *et al.*, 2009; ARTERO *et al.*, 2012; CHUNG *et al.*, 2014; VOLAKLIS *et al.*, 2015a). Outro componente importante no curso da doença são os níveis elevados de marcadores pró-inflamatórios, como por exemplo o Fator de Necrose Tumoral Alfa (TNF-α), que tem demonstrado associação com o quadro geral da IC e observa-se também a existência de múltiplos efeitos biológicos que impactam na função da mecânica cardíaca (PRABHU, 2004), edema pulmonar (YANG *et al.*, 2010) e remodelamento ventricular (OIKONOMOU *et al.*, 2011).

A elevação das citocinas pró-inflamatórias tem sido relacionada com a redução de massa e força muscular (ZHOU *et al.*, 2016b) e também com uma reduzida capacidade funcional (TORRE-AMIONE *et al.*, 1996). Por outro lado, o exercício físico tem ajudado a inverter esse cenário deletério imposto pela síndrome da IC (NIEBAUER, 2008; DOWNING & BALADY, 2011; SMART & STEELE, 2011).

Lesões no músculo cardíaco levam a alterações na matriz extracelular e consequentemente, alteram o tamanho, a forma e a função do ventrículo, resultando no processo chamado de remodelamento cardíaco (MCMURRAY, 2010). As alterações na matriz extracelular do colágeno cardíaco estão associadas com prejuízos na complacência cardíaca, na função valvular, na função sistólica e diastólica e no comportamento elétrico do coração (WHITTAKER *et al.*, 1991; YU *et*

al., 1996; VAN PUTTEN *et al.*, 2016). Interessantemente, em trabalhos que utilizaram animais, o uso do exercício físico tem apresentado respostas favoráveis sobre as frações de colágeno intersticiais (VANZELLI *et al.*, 2013; ALMEIDA *et al.*, 2014; ALVES *et al.*, 2014; DE MORAES *et al.*, 2015; NUNES *et al.*, 2015). De uma forma geral o exercício físico tem sido apontado como uma ferramenta efetiva para proporcionar o remodelamento reverso cardíaco frente a fisiopatologia imposta pela IC (ADAMS & NIEBAUER, 2015).

Nos últimos anos, várias metodologias baseadas em exercício físico têm sido testadas como potentes ferramentas não farmacológicas tanto em pacientes com IC (WISLOFF *et al.*, 2007; TOTH *et al.*, 2012; BENDA *et al.*, 2015) como no modelo experimental de infarto do miocárdio (IM) (BATISTA *et al.*, 2010; ALVES *et al.*, 2014; NUNES *et al.*, 2015). O uso do TF no modelo experimental com ratos saudáveis, demonstrou ser uma ferramenta efetiva para adaptações positivas na função cardíaca, no remodelamento cardiovascular (BARAUNA *et al.*, 2005; BARAUNA *et al.*, 2007) e no músculo esquelético (TAMAKI *et al.*, 1992). Atualmente, o protocolo de TF adaptado para ratos tem demonstrado respostas positivas em animais com diabetes, pois é observado uma recuperação da função cardiovascular nesse modelo experimental (MOSTARDA *et al.*, 2014). Em ratos após o IM também foram observadas respostas positivas frente a grave disfunção ventricular esquerda após oito semanas de TF de moderada intensidade (ALVES *et al.*, 2014).

Os efeitos do treinamento físico, praticados de forma isolada ou combinada, sobre a função diastólica, precisam de melhores esclarecimentos em relação ao tipo, frequência e intensidade do exercício físico (FONTES-CARVALHO *et al.*, 2015a). Os estudos em modelos animais de IC têm utilizado, na sua maioria, o protocolo de treinamento aeróbico contínuo (TAC) (NUNES *et al.*, 2008b; VANZELLI *et al.*, 2013; ALMEIDA *et al.*, 2014; DE MORAES *et al.*, 2015; NUNES *et al.*, 2015; GUIZONI *et al.*, 2016). Portanto, hipotetizamos que o TF de alta intensidade combinado com o TAC de moderada intensidade pode ter efeitos superiores ao TF isolado ou TAC isolado sobre o ganho de força e capacidade funcional e essas respostas podem estar associadas com a melhora da função hemodinâmica, do perfil inflamatório e da deposição de colágeno em animais com IC. Também acreditamos que as variáveis de volume e intensidade do protocolo de agachamento para ratos no modelo experimental de IC e em ratos saudáveis podem modular positivamente as

respostas do ganho de força e os consequentes desfechos associados à disfunção ventricular esquerda e volume de colágeno.

1.1 Reabilitação cardíaca e a inserção do exercício físico

A biologia do envelhecimento e a fisiopatologia das doenças cardiovasculares (DCV) se sobrepõem e atualmente as DCV tornaram-se endêmicas na crescente população de adultos mais velhos (SCHOPFER & FORMAN, 2016). As DCV são a principal causa de morte no mundo, somando atualmente 17,3 milhões de mortes por ano, sendo que esses números podem aumentar para mais do que 23,6 milhões de mortes no mundo para o ano de 2030 (MOZAFFARIAN *et al.*, 2015).

Dentre as DCV podemos destacar a IC que é altamente prevalente em indivíduos mais velhos e é a maior causa de morbidade, mortalidade, hospitalizações e incapacidade (ADES *et al.*, 2013). No Brasil a IC tem apresentado uma alta taxa de mortalidade; um estudo recente, liderado por Albuquerque e colaboradores (2015) demonstrou que 50 mil brasileiros morrem por ano devido a essa síndrome complexa (ALBUQUERQUE *et al.*, 2015).

A IC é um problema de saúde endêmico no mundo ocidental e, apesar dos grandes esforços dos profissionais da área da saúde, durante a última década observa-se que a morbidade e a mortalidade continuam muito elevadas (MARIN-GARCIA *et al.*, 2012). A síndrome da IC foi considerada, por muito tempo, uma doença incurável e com poucas perspectivas em relação a sua recuperação. No final do século XX, os avanços no campo da ciência permitiram um melhor entendimento da fisiopatologia da IC e sua repercussão sistêmica, envolvendo principalmente alterações hemodinâmicas e ativação neuro-humoral, juntamente com o desenvolvimento de tratamentos eficazes, o que fez com que passasse a ser considerada uma doença crônica (MUDD & KASS, 2008).

A restrição de atividades físicas para os pacientes com IC foi recomendada até o final das décadas de 1970 e 1980, pois era reconhecido que a capacidade ao exercício, em pacientes com disfunção ventricular esquerda, não poderia ser predita por tais parâmetros como pressão de enchimento intracardíaco e fração de ejeção ventricular esquerda (THOMPSON *et al.*, 2003). Além disso, a hipertrofia cardíaca patológica, redução na função sistólica ventricular esquerda e juntamente com a

própria disfunção cardíaca foram consideradas contraindicações absolutas ou relativas para a realização do treinamento físico (WORKING GROUP ON CARDIAC *et al.*, 2001).

Atualmente as evidências científicas têm demonstrado que o exercício físico pode ser uma ferramenta efetiva para o tratamento adequado de pacientes com DCV e inúmeros estudos têm justificado a utilização de variadas formas do exercício físico frente ao cenário deletério que a IC impõe (BENDA *et al.*, 2015; FONTES-CARVALHO *et al.*, 2015b; YAMAMOTO *et al.*, 2016). O exercício físico ganhou destaque nos últimos anos, pois observa-se respostas favoráveis sobre a capacidade funcional, qualidade de vida e redução de mortalidade em pacientes com DCV que aderem à prática de exercícios aeróbicos ou resistidos (JANKOWSKA *et al.*, 2008; SAVAGE *et al.*, 2011; DE MAEYER *et al.*, 2013).

Dentre as principais propostas para a prática do exercício físico em um programa de reabilitação cardíaca (RC) o TA é o mais utilizado de longa data e tornou-se uma ferramenta segura e efetiva para pacientes com doenças cardiovasculares, tal modalidade tem sido amplamente utilizada há mais de 40 anos (BJARNASON-WEHRENS *et al.*, 2004). Recentemente, outras modalidades de treinamento físico começaram a fazer parte dos programas de RC, com destaque especial para o TF, o qual tem sido investigado nos últimos anos (VOLAKLIS & TOKMAKIDIS, 2005; SAVAGE *et al.*, 2011; TOTH *et al.*, 2012; VOLAKLIS *et al.*, 2015b).

As diretrizes da *American Heart Association* (AHA) recomendam que o TA e o TF são os componentes centrais de um programa de RC. Interessantemente, em uma meta-análise, Cochrane demonstrou que a combinação do TA com o TF foi associada a uma redução de 28% na mortalidade e uma redução de 31% na readmissão hospitalar em pacientes com doença arterial coronariana (HERAN *et al.*, 2011).

A literatura científica tem sugerido que as propostas baseadas em intervenções combinadas podem ser as estratégias mais efetivas para o tratamento das DCV, pois cada proposta apresenta respostas específicas em diferentes áreas frente à complexidade do cenário fisiopatológico da IC (BOUCHLA *et al.*, 2011; ADAMOPOULOS *et al.*, 2014). Portanto, dentro dessa linha de raciocínio, um grupo de pesquisadores gregos realizou uma proposta com três diferentes intervenções

utilizadas no formato combinado e o grupo fez a seguinte pergunta: *Benefits of combined aerobic/resistance/inspiratory training in patients with chronic heart failure. A complete exercise model? A prospective randomised study* (LAOUTARIS *et al.*, 2013). Talvez a resposta fique por conta do quanto as metodologias combinadas possam ser factíveis de serem realizadas na população com DCV e possivelmente existirão diferentes desfechos para diferentes frequências, intensidades e volumes das estratégias terapêuticas aplicadas. Além disso, o avanço no quadro da doença pode ser um fator limitante para a prática de determinados protocolos.

1.2 Exercício aeróbico na insuficiência cardíaca

A IC pode ser definida como a inabilidade do coração em atender as demandas teciduais, o que, consequentemente, resulta em sintomas como fadiga ou dispneia em esforço, progredindo para dispneia em repouso. Essa incapacidade de realizar o esforço físico sem desconforto pode ser um dos primeiros sintomas que acometem os pacientes com IC e, frequentemente, essa é a principal razão pela busca de cuidados médicos (PINA *et al.*, 2003).

A capacidade para a realização do exercício aeróbico depende da habilidade do coração em aumentar o débito cardíaco para os músculos em exercício e da habilidade desses músculos em utilizar o oxigênio a partir do suporte sanguíneo entregue (PINA *et al.*, 2003). Alguns estudos da década de 90 demonstraram que o exercício físico pode levar a um significante aumento na capacidade aeróbica muscular, juntamente com um aumento acentuado na área de secção transversa de fibras musculares, densidade mitocondrial, expressão da citocromo C oxidase e densidade capilar em pacientes com IC (BELARDINELLI *et al.*, 1995; HAMBRECHT *et al.*, 1995).

Em 2012, uma diretriz europeia para diagnóstico e tratamento de insuficiência cardíaca incorporou como classe de evidência IA a recomendação para a prática regular de exercício aeróbico em pacientes com IC para melhorar a capacidade funcional e sintomas decorrentes da doença (MCMURRAY *et al.*, 2012). A inserção da prática do TA para pacientes com IC torna-se de extrema importância, pois tal modalidade apresenta benefícios como: redução da intolerância ao exercício físico, melhora da função diastólica, melhora da função endotelial, aumento da capacidade

oxidativa muscular, aumento do tônus vagal e redução do tônus simpático, redução das citocinas pró inflamatórias, redução por toda a causa de mortalidade e tempo de hospitalização, sendo que tais respostas culminam na melhora do *status* de saúde do paciente com IC (DOWNING & BALADY, 2011).

Adicionalmente, dentro do quadro da IC, a ausência de melhora na capacidade de realizar o exercício físico, após um programa de treinamento físico, apresenta um forte valor prognóstico para eventos cardíacos, independente da fração de ejeção ventricular esquerda (FEVE), classificação do nível da doença segundo a *New York Heart Association* (NYHA) e níveis do Peptídeo Natriurético Tipo-B (TABET *et al*., 2008). Em adição, o VO_{2pico} pode ser considerado um determinante de sobrevida em pacientes com IC, pois uma redução no consumo do VO_{2pico} (consumo máximo de oxigênio atingido antes de ocorrer estabilização da quantidade de oxigênio captado) pode ser um preditor importante em pacientes que recebem terapia farmacológica com β-bloqueador quando comparado com aqueles pacientes tratados sem este medicamento (O'NEILL *et al.*, 2005).

Respostas positivas por meio da exposição ao exercício aeróbico no modelo experimental de IM são bem sustentadas na literatura, pois algumas investigações demonstraram que a execução desse protocolo aeróbico pode melhorar alguns parâmetros como: hemodinâmica central, perfil inflamatório, deposição de colágeno cardíaco e congestão pulmonar (NUNES *et al.*, 2008b; BATISTA *et al.*, 2010; NUNES *et al.*, 2015). Além disso, também são observadas respostas positivas em relação a atenuação da disfunção cardíaca, acompanhada pela prevenção da atrofia muscular esquelética, a qual está associada com uma atividade anti-catabólica presumidamente causada pela ação da *Peroxisome proliferator-activated receptor-gamma coactivator* (PGC1-α) (SOUZA *et al.*, 2014).

1.3 Treinamento de força e insuficiência cardíaca

Alguns estudos têm sugerido que o TF complementar, quando bem prescrito e supervisionado, tem efeitos favoráveis sobre a força e a resistência muscular, a função cardiovascular, o metabolismo, os fatores de risco coronariano e o bem-estar psicossocial (POLLOCK *et al.*, 2000). Além disso, essa modalidade de treinamento

físico previne o declínio da massa muscular esquelética, preservando sua função (HURLEY & ROTH, 2000).

Algumas investigações têm recomendado a inclusão do TF ao condicionamento aeróbico para maximizar os efeitos benéficos do treinamento, pois o TF fornece benefícios complementares ao TA, que inclui preservação da massa muscular magra, aumento do conteúdo mineral ósseo, redução de quedas e aumento da qualidade de vida, logo proporcionando uma melhor execução das atividades de vida diária (MAIORANA *et al.*, 2000; GARY *et al.*, 2011).

O TF prescrito com intensidade adequada é relacionado a um menor estresse hemodinâmico do que a grande maioria das prescrições baseadas no TA, podendo, ainda, ter uma vantagem adicional devido a sua especificidade que é reduzir as limitações periféricas, característica comum encontrada em pacientes com IC (CHEETHAM *et al.*, 2002).

Interessantemente, o TF pode melhorar o torque isométrico em pacientes com IC, mas não altera a área de secção transversa do músculo ou o tamanho da fibra muscular. Tais respostas estão associadas com eventos celulares e moleculares da adaptação ao TF, pois é observado melhora na cinética das pontes cruzadas, como por exemplo: maior tempo de conexão da miosina em pacientes com IC submetidos ao TF. Essas respostas indicam um efeito corretivo do TF sobre a mecânica da contração muscular (TOTH *et al.*, 2012).

O TF pode modificar positivamente a classificação da gravidade da IC. Tal resposta é observada através do *status* clínico desses pacientes no final de um programa de treinamento, como relatado por Jankawska e colaboradores através de uma investigação muito bem planejada, na qual os pacientes submetidos ao TF passaram da classificação III/NYHA para II/NYHA. Além disso, também foi observado que no período de destreinamento (12 semanas após o treinamento) poucos pacientes retornaram para a classificação mais elevada da doença. Esses achados refletiram na melhora da qualidade de vida que também foi um desfecho importante dessa investigação (JANKOWSKA *et al.*, 2008).

O TF pode influenciar positivamente na capacidade funcional e nas atividades de vida diária de pacientes com IC através de um protocolo de TF. Tais respostas demonstram que o treinamento de força é capaz de reverter a incapacidade física gerada pela doença (SAVAGE *et al.*, 2011). Outros efeitos favoráveis são

apresentados através das respostas benéficas sobre o remodelamento arterial, como demonstrado pelo estudo de Maiorana e colaboradores. Nessa investigação, o grupo submetido ao TF apresentou respostas mais pronunciadas quando comparado ao grupo do TA, uma vez que a espessura da parede do vaso e a razão parede/lúmen apresentaram modificações positivas somente no grupo submetido ao TF (MAIORANA *et al.*, 2011). No entanto, mais evidências são necessárias sobre os efeitos do TF em pacientes com IC, pois a literatura ainda não fornece indícios claros de que o TF de moderada ou alta intensidade é estatisticamente e clinicamente significativo em relação aos efeitos positivos desta modalidade de treinamento (SPRUIT *et al.*, 2009).

1.4 Modelo experimental de infarto do miocárdio

O modelo experimental de IM após a ligadura da artéria coronária em ratos é uma técnica utilizada há mais de 30 anos (PFEFFER *et al.*, 1979; KLOCKE *et al.*, 2007). A oclusão da artéria coronária descendente esquerda leva à isquemia miocárdica crônica e, consequentemente, à IC. A oclusão completa resulta em infarto do miocárdio com ocorrência evidente de IC após 3 a 6 semanas (HASENFUSS, 1998; FRANCIS, 2001).

A ligadura da artéria coronária esquerda promove alterações na hemodinâmica, uma vez que a necrose do tecido miocárdico leva a um quadro de disfunção ventricular esquerda, e esta alteração está diretamente relacionada ao tamanho da área infartada (PFEFFER *et al.*, 1979; FRANCIS, 2001; CHIMENTI *et al.*, 2004). Neste modelo de indução da IC, áreas maiores que 40% (Figura 1), representam uma perda grave do miocárdio, o que resulta em disfunção hemodinâmica sustentada com pressão diastólica final do ventrículo esquerdo (PDFVE) maiores que 20 mmHg (Figura 2), menor pressão sistólica ventricular esquerda (PSVE) e hipertrofia cardíaca, reproduzindo um quadro de IC grave (PFEFFER *et al.*, 1979; FRANCIS, 2001; MUSCH *et al.*, 2002; NUNES *et al.*, 2008b; ALVES *et al.*, 2014; CAPALONGA *et al.*, 2016).

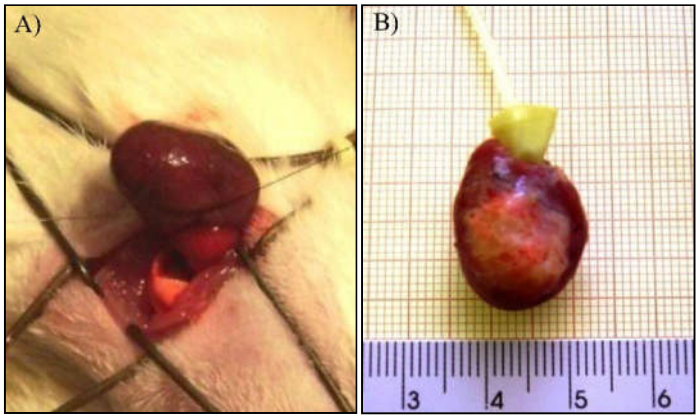

Figura 1. Área de infarto provocada por IM através da ligadura da artéria coronária. Ligadura da artéria coronária esquerda; B) Cicatriz fibrótica da área de infarto de 40% da área total do ventrículo esquerdo. Imagens cedidas pelo Laboratório de Fisiologia –UFCSPA.

Figura 2. Ondas de pressão ventricular com aumento da PDFVE. Ondas de pressão ventricular esquerda registrada por CODAS; A) Rato sham com PDFVE normal (1,66mmHg); B) Rato com IC 14 semanas pós IM (25,06mmHg). Imagens cedidas pelo Laboratório de Fisiologia –UFCSPA.

Os primeiros estudos relacionados ao modelo experimental com indução de IM em pequenos animais foram descritos há algumas décadas por Heimburger e

colaboradores (1946) e Johns & Olson (1954) (HEIMBURGER, 1946; JOHNS & OLSON, 1954), mas o conhecimento mais detalhado sobre as modificações hemodinâmicas causadas pela área de necrose miocárdica foi melhor descrita por Pfeffer e colaboradores (1979) (PFEFFER *et al.*, 1979). Neste estudo, após a indução de IM e o consequente desenvolvimento de IC em ratos, foi observado aumento na PDFVE e do peso do ventrículo direito, consistentes com a elevação da pressão da artéria pulmonar devido à indução da insuficiência cardíaca ventricular esquerda, caracterizando o modelo de IC crônica; também foram observadas reduções na pressão arterial média (PAM) e pressão arterial sistólica (PAS) no grupo com grande área de infarto (PFEFFER *et al.*, 1979).

O uso de ratos Wistar para o desenvolvimento de IC experimental pós IM parece ser uma boa opção para investigar a fisiopatologia da IC, pois esse modelo reproduz as alterações cardiovasculares, músculo-esqueléticas e metabólicas, desta síndrome. Dessa forma, possibilita o estudo da efetividade de diferentes intervenções terapêuticas como, por exemplo, os efeitos do exercício físico sobre a função hemodinâmica e o perfil inflamatório (NUNES *et al.*, 2008a), os efeitos do treinamento muscular ventilatório sobre a mecânica ventilatória (JAENISCH *et al.*, 2011), os efeitos da eletroestimulação sobre a expressão de GLUT-4 e densidade capilar da musculatura esquelética (DE LEON *et al.*, 2011) e os efeitos da laserterapia sobre a inflamação local do músculo esquelético (HENTSCHKE *et al.*, 2012).

O modelo experimental de IC assemelha-se à síndrome crônica observada durante a IC em humanos (FRANCIS, 2001), permitindo uma alternativa importante para o estudo mecanístico, bem como para a compreensão do cenário patológico da IC. No entanto, é reconhecido que a complexidade das doenças humanas que levam a IC representa uma importante barreira a ser transposta, pois muitas das alterações sistêmicas são difíceis de serem mimetizadas na maioria dos modelos animais (HOUSER *et al.*, 2012), ficando subentendido que nenhum método reproduz perfeitamente a condição de saúde prévia que normalmente está associada ao surgimento da IC. Todavia, apesar das limitações pertinentes ao modelo, os estudos experimentais com uso de animais de laboratório permanecem sendo uma das melhores ferramentas para avançar no entendimento das DCV (ZARAGOZA *et al.*, 2011).

1.5 Remodelamento cardíaco na insuficiência cardíaca

Os mecanismos existentes para explicar as alterações do músculo cardíaco são complexos, uma vez que, após o IM, modificações estruturais e bioquímicas decorrentes desse evento resultam em efeitos deletérios sobre a capacidade funcional do coração, progredindo então ao quadro da IC (COHN *et al.*, 2000). O remodelamento ventricular é um acurado preditor de mortalidade cardíaca seguido ao IM (GIALLAURIA *et al.*, 2008).

Após o IM, o músculo cardíaco sofre modificações estruturais e consequentemente funcionais, tal fenômeno pode ser chamado de remodelamento cardíaco, mais precisamente de hipertrofia patológica, a qual ocorre através da modulação da função do fibroblasto cardíaco, principal componente da matriz extracelular, que está envolvido em múltiplas respostas ligadas ao remodelamento cardíaco, como por exemplo: diferenciação, migração e proliferação dos miofibroblastos, os quais estão envolvidos na interferência da propagação dos sinais elétricos que controlam o ritmo cardíaco (WHITTAKER, 1995; PORTER & TURNER, 2009; LOPEZ *et al.*, 2016).

A resistência relativa dos fibroblastos, decorrente da hipóxia e a sua notável plasticidade adaptativa em resposta às rápidas mudanças no microambiente do tecido local, determinam que o fibroblasto cardíaco intersticial desempenha um papel chave após o IM durante o período de reparação tecidual, essa população de células é mobilizada rapidamente em resposta à lesão cardíaca (CHISTIAKOV *et al.*, 2016). Os fibroblastos também estão envolvidos no aumento de citocinas pró-inflamatórias e nos fatores de crescimento e esses eventos alteram diretamente a síntese e degradação da matriz extracelular, principalmente pelo aumento das metaloproteínases (MMP) e redução dos fatores de inibição das metaloproteínases (TIMP) (SPINALE, 2007; PORTER & TURNER, 2009).

Recentemente, foi demonstrado que o desequilíbrio entre as ações de síntese e degradação do colágeno intersticial em pacientes com IC de etiologia hipertensiva está associado com valor prognóstico e hospitalização, demonstrando que o acúmulo de colágeno tem um papel crucial na função cardíaca desses pacientes que apresentam comprometimento da função sistólica e diastólica (LOPEZ *et al.*, 2016).

O estresse gerado sobre o fibroblasto cardíaco leva a um ciclo vicioso de eventos que estimulam a ativação do miofibroblasto, que por sua vez induz o aumento da fibrose miocárdica que está intimamente relacionada ao aumento da rigidez da câmara cardíaca, arritmias e prejuízo na oferta de nutrientes e oxigênio para o tecido cardíaco, consequentemente se desenvolve a hipertrofia do cardiomiócito resultando em morte tecidual e todo o ciclo do processo inflamatório e apoptose celular é repetido (PIEK *et al.*, 2016).

Esse mecanismo de síntese e degradação da matriz extracelular é responsável pelo aumento de colágeno cardíaco, principalmente o tipo I e III, tanto em estudos com humanos, como em modelos animais que têm demonstrado que o acúmulo de colágeno está associado com prejuízos na complacência ventricular (ALVES *et al.*, 2014; VAN PUTTEN *et al.*, 2016).

Adicionalmente, o remodelamento cardíaco pode liberar as cardiocinas pró-inflamatórias (processo inflamatório decorrente da adaptação do tecido cardíaco), tais como IL-1β e IL-18, esse processo pode fortemente impactar em dano aos tecidos periféricos como: baço, rim, músculo esquelético e tecido adiposo, esse quadro piora paralelamente com o grau e a duração da injúria do tecido miocárdico (JAHNG *et al.*, 2016).

A hipoperfusão causada pelo evento isquêmico no coração apresenta alterações nos mecanismos celulares e moleculares o que, consequentemente, estimula o remodelamento cardíaco adverso (GAJARSA & KLONER, 2011), tal fenômeno pode produzir alterações tanto na anatomia da região infartada como na região não infartada do ventrículo e essas modificações tendem a afetar a função ventricular e o prognóstico de sobrevida (PFEFFER & BRAUNWALD, 1990). Por outro lado, a prática de exercício físico também proporciona adaptações ao músculo cardíaco, causando um estado fisiológico de remodelamento cardíaco, mas estas mudanças morfológicas podem diferir, dependendo do tipo de treinamento, e são clinicamente caracterizadas por modificações no tamanho e forma cardíaca devido ao aumento da carga (MIHL *et al.*, 2008).

Os efeitos do exercício físico aeróbico ou resistido no modelo experimental de IM têm demonstrado respostas favoráveis sobre a deposição de colágeno no interstício do miocárdio (XU *et al.*, 2008; ALMEIDA *et al.*, 2014; ALVES *et al.*, 2014), esses estudos demonstraram que animais após o IM, quando submetidos à prática

do exercício físico, podem reorganizar de forma positiva o acúmulo de fibrose no coração infartado. Recentemente, foi demonstrado que o TA em animais com IM pode alterar de forma benéfica o tamanho da câmara cardíaca, esse desfecho foi relatado através da avaliação por ressonância magnética (IZELI *et al.*, 2016).

1.6 Repercussões sistêmicas da Insuficiência Cardíaca

Após o IM, a disfunção diastólica é frequente e também é um importante determinante de respostas adversas (FONTES-CARVALHO *et al.*, 2015a). A disfunção diastólica é prevalente na população e é reconhecida como um importante preditor de IC e mortalidade de longo prazo (REDFIELD *et al.*, 2003; FITZGIBBONS *et al.*, 2009). Por outro lado, também é observado que a IC pode ocorrer com uma reduzida fração de ejeção ou preservada fração de ejeção (HAMMOND *et al.*, 2016).

As primeiras anormalidades observadas em pacientes com IC sistólica são a reduzida reserva contrátil do ventrículo esquerdo, causando diminuição do volume sistólico e no débito cardíaco (SHAMMAS *et al.*, 2007), o que promove, neste cenário fisiopatológico, a incapacidade do coração de bombear adequado volume sanguíneo requerido pela atividade metabólica tecidual (LIBBY, 2008). Recentemente, um estudo de seguimento de longo prazo, demonstrou que pacientes com diagnóstico de insuficiência cardíaca sistólica têm pior prognóstico de mortalidade quando comparados com pacientes que têm IC com disfunção diastólica (OLOFSSON & BOMAN, 2015).

O conhecimento sobre os fatores que modulam a IC tem evoluído e a complexidade global da doença tornou-se mais evidente, tornando claro que os mecanismos periféricos da doença, particularmente aqueles relacionados com a perfusão dos tecidos e o metabolismo oxidativo do músculo esquelético, geram um impacto importante na progressão da IC. Ademais, criam um potencial cenário para novas intervenções terapêuticas, como, por exemplo, o exercício físico, que tem demonstrado ser uma estratégia para melhorar a musculatura esquelética e vascular dos pacientes com IC (DUSCHA *et al.*, 2008).

Entre as alterações sistêmicas relacionadas ao quadro progressivo da IC, são observadas modificações na hemodinâmica e ativação inflamatória sistêmica com aumento evidente na expressão gênica e produção de citocinas pró-inflamatórias,

como o TNF-α, interleucina 1β (IL-1 β) e interleucina 6 (IL-6), tanto no plasma como no músculo esquelético (SCHULZE *et al.*, 2003; ANKER & VON HAEHLING, 2004; YNDESTAD *et al.*, 2006). Além de cooperar com a fisiopatologia e a progressão da injúria estrutural e funcional do miocárdio, estes mediadores inflamatórios podem atuar diretamente no surgimento das manifestações periféricas da síndrome da IC, principalmente nas modificações relacionadas à redução da massa muscular e diminuição da capacidade funcional (GIELEN et al., 2003; TOTH et al., 2006).

O desequilíbrio entre os mediadores inflamatórios tem sido relacionado com prejuízos tanto sistêmicos como no tecido cardíaco, contribuindo negativamente para a progressão da síndrome da IC. Atualmente, é observado que as citocinas pro-inflamatórias têm uma ampla ação sobre a função sistólica, disfunção endotelial, ativação do programa gene fetal, depressão (emoção), caquexia, anemia, tromboembolismo, hipertrofia do miocárdio, apoptose de células miocárdicas, distúrbios da matriz extracelular miocárdica e efeito inotrópico negativo (OIKONOMOU *et al.*, 2011; GULLESTAD *et al.*, 2012).

As citocinas inflamatórias como TNF-α e IL-6 desempenham um importante papel no desenvolvimento da caquexia. Estas citocinas induzem atrofia muscular esquelética pela ativação da via da miostatina e ubiquitina proteassoma, ou pela inibição da síntese proteica muscular e biogênese mitocondrial. Frente a esse quadro, o exercício físico tem sido proposto por apresentar benefícios adicionais quando comparado com outros modelos de terapias, pois a inserção dessa ferramenta não farmacológica além de estimular a síntese de proteínas também inibe o processo inflamatório (ZHOU *et al.*, 2016b).

Outro componente sistêmico e importante é a espessura da parede do vaso e a razão parede/lúmen que têm sido utilizados para refletir o remodelamento arterial. Esse processo pode ser influenciado pelas respostas do exercício físico sobre o sistema hemodinâmico através do componente de *shear stress* (DINENNO *et al.*, 2001; MOREAU *et al.*, 2006; TINKEN *et al.*, 2010; MAIORANA *et al.*, 2011). Reduzida espessura da parede da artéria braquial está associada com a redução no risco de eventos cardiovasculares, ao passo que o aumento progressivo na espessura da parede da artéria braquial aumenta paralelamente a gravidade nos casos de IC (FRICK *et al.*, 2005; POELZL *et al.*, 2005).

Durante o curso da IC, a bioenergética é alterada negativamente, pois todas as etapas de extração, transferência e utilização de energia são afetadas e o metabolismo estrutural é prejudicado, esse desequilíbrio energético leva a um comprometimento da integridade funcional dos tecidos, ocorrendo não apenas no miocárdio, mas também na falha metabólica de tecidos e órgãos, resultando em um global desequilíbrio dos sinais anabólicos e catabólicos, desencadeando perda tecidual o que leva consequentemente ao estado de caquexia (DOEHNER *et al.*, 2014).

1.7 Fraqueza muscular e insuficiência cardíaca

Com o aumento da expectativa de vida, a perda progressiva de massa muscular associada ao processo de envelhecimento está se tornando um grande problema para a área da saúde pública (EBNER *et al.*, 2015). O prejuízo no sistema músculo esquelético desencadeia uma evidente redução na força muscular, esse panorama é um fenômeno comum que leva a uma ampla variedade de distúrbios associados com o envelhecimento e estimulam algumas condições catabólicas associadas com o as morbidades, tal como a IC crônica (EBNER *et al.*, 2015). A IC é uma condição patológica que causa fraqueza muscular sistêmica através do estado catabólico estimulado pela ativação da miostatina e resistência à insulina (COHEN *et al.*, 2015). Além disso, a partir da década de 90, a perda de massa muscular e a fraqueza muscular passaram a ser relacionadas com o aumento da mortalidade em pacientes com IC, também foi observado que o aumento da força muscular é um importante fator para aumentar a sobrevida desses pacientes (ANKER *et al.*, 1997; HULSMANN *et al.*, 2004).

A compreensão dos mecanismos da perda da massa muscular e da redução da força muscular na IC são imprescindíveis para uma adequada prescrição do exercício físico baseada em evidências, uma vez que as anormalidades na musculatura esquelética podem ser classificadas dentro de dois tipos: 1) Baixa resistência aeróbica decorrente da disfunção mitocondrial; e 2) Perda de massa muscular e força muscular devido ao desequilíbrio entre síntese e degradação proteica (KINUGAWA *et al.*, 2015). Adicionalmente, outros fatores complexos do

processo inflamatório também contribuem para esse cenário deletério sobre a função da massa muscular esquelética em pacientes com IC (ZHOU *et al.*, 2016a).

O desequilíbrio entre anabolismo e catabolismo muscular leva os pacientes com IC a uma reduzida capacidade ao exercício, a qual limita as atividades básicas de vida diária e consequentemente estimula um grande prejuízo para a qualidade de vida desses pacientes (KINUGAWA *et al.*, 2015). Além disso, a reduzida capacidade ao exercício está diretamente relacionada com a gravidade e o prognóstico da IC, pois as anormalidades na musculatura esquelética apresentam um cenário de prejuízos como: metabolismo energético desequilibrado, transição das fibras do tipos I para ao tipo II, disfunção mitocondrial, redução na força muscular e atrofia muscular (MILLER *et al.*, 2009; GEORGIADOU & ADAMOPOULOS, 2012; ZIZOLA & SCHULZE, 2013; KINUGAWA *et al.*, 2015).

Alguns mecanismos moleculares têm sido amplamente investigados nos últimos anos para o melhor entendimento sobre o fenômeno da perda de massa muscular e possíveis alternativas para reversão desse quadro, não somente na IC, mas também na doença pulmonar obstrutiva crônica, diabetes, insuficiência renal e câncer associado com caquexia (COHEN *et al.*, 2015). Dentre as vias de sinalizações moleculares relacionadas com a perda da massa muscular, no cenário das doenças crônicas, é observada uma redução na sinalização da via phosphoinositide 3-kinase PI3K-AKT-mTOR que leva a falha na síntese de proteínas, elevando drasticamente a proteólise através da aumentada expressão da *forkhead box protein O* (FOXO) mediada pela transcrição atrogene (LATRES *et al.*, 2005). Essa cascata de eventos moleculares é suficiente para induzir a proteólise através da ativação da ubiquitina proteassoma e autofagia, consequentemente estimulando a atrofia muscular (SANDRI *et al.*, 2004; MAMMUCARI *et al.*, 2007; COHEN *et al.*, 2015).

A atrofia muscular esquelética induzida pela IC está associada com uma sequência de eventos degradadores, como por exemplo: aumento da expressão da NOX2, produção de espécies reativas ao oxigênio (ERO) e ativação do NF-kβ, o que leva ao aumento da expressão da MuRF-1 (componente do sistema da ubiquitina-proteossoma envolvido na proteólise muscular) e ativação da proteossoma (BECHARA *et al.*, 2014). Por outro lado, o exercício físico aeróbico tem demonstrado efeitos favoráveis sobre esse sistema complexo, pois é observado uma redução nos

níveis de MuRF-1 após quatro semanas de treinamento, sugerindo que uma ativação no bloqueio do sistema ubiquitina-proteossoma pode ocorrer em pacientes com IC independentemente da idade, como demonstrado elegantemente por Gielen e colaboradores (GIELEN *et al.*, 2012).

Atualmente, sabe-se que a PGC-1α é um coativador de transcrição induzido pelo exercício aeróbico que favorece adaptações positivas na musculatura esquelética relacionadas com a melhora da resistência, mas não tem efeitos sobre a força muscular ou hipertrofia (RUAS *et al.*, 2012). Entretanto, uma isoforma da PGC-1α, conhecida como PGC-1α4, é altamente expressada no músculo esquelético através do TF e não tem ação reguladora nos genes alvos mitocondriais da PGC-1α. Pelo contrário, a PGC-1α4 induz especificamente o fator de crescimento semelhante à insulina tipo 1 (IGF-1) e inibe a ação da miostatina, consequentemente a expressão da PGC-1α4 favorece uma robusta hipertrofia muscular esquelética demonstrada tanto *in vitro* como *in vivo* (RUAS *et al.*, 2012).

A indicação do exercício físico tanto aeróbio como resistido para pacientes com IC tem sido apontada como uma estratégia efetiva para devolver o equilíbrio entre degradação e síntese proteica durante o curso da doença, visto que é observado que as sinalizações moleculares deletérias como aumento da atividade da ubiquitina proteassoma e consequentemente ativação da MuRF-1 (atividade proteolítica) podem ser inibidas através de intervenções baseadas da prática do exercício físico e, dessa forma, reestabelecer a capacidade funcional da musculatura esquelética (GIELEN *et al.*, 2012; RUAS *et al.*, 2012; KINUGAWA *et al.*, 2015).

Além disso, a combinação do TF com o TA tem demonstrado ser mais efetiva para aumento da expressão de PGC-1α e sua isoforma PGC-1α4, também é observado uma redução na expressão do gene da miostatina quando se utiliza o protocolo de TF isolado ou cominado ao TA, essas respostas demonstram que o exercício físico tem uma ação positiva e intrínseca na musculatura esquelética ajudando na melhora da função muscular (RUAS *et al.*, 2012).

1.8 Modelo experimental de agachamento adaptado para roedores

O modelo experimental de TF é um método relativamente novo, visto que os primeiros artigos com este tipo de protocolo foram publicados no início da década de

90 (TAMAKI *et al.*, 1992) e nos últimos anos tem sido utilizado como modelo de investigação nos mais variados cenários (NUNES *et al.*, 2013; ALVES *et al.*, 2014; MOSTARDA *et al.*, 2014; STEFANI *et al.*, 2014; NEVES *et al.*, 2016). Com o passar dos anos, foram desenvolvidos alguns tipos de protocolos para induzir a hipertrofia muscular nesses animais, tal tentativa tem por objetivo mimetizar as adaptações que ocorrem em seres humanos submetidos ao TF (LOWE & ALWAY, 2002).

Para um melhor entendimento sobre o tema, apresentamos nessa referente tese alguns modelos que contribuíram no avanço dos protocolos de TF para modelos animais, pois esses protocolos já foram utilizados em variadas metodologias e logo abaixo pode-se observar alguns tipos de protocolos e suas evoluções:

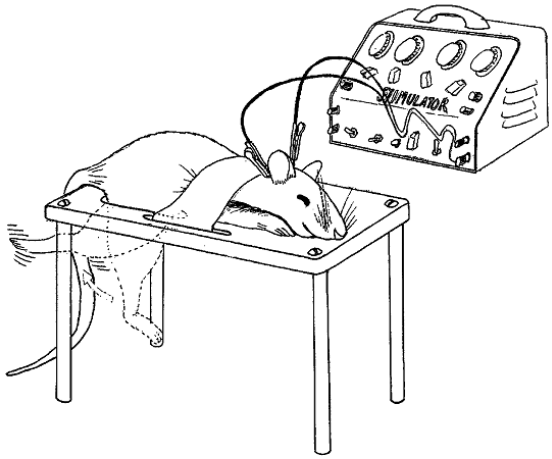

Figura 3 - Modelo experimental de indução de hipertrofia muscular por estimulação elétrica.

Nesse estudo, os investigadores usaram eletrodos invasivos ligados ao nervo ciático dos animais para produzir contrações de alta resistência, após o estímulo elétrico os animais movimentavam suas patas traseiras como ilustrado na figura acima (BAAR & ESSER, 1999).

Figura 4 – Modelo de treinamento físico pelo método de escalada.

Na figura 4 é ilustrado o protocolo de escalada, pode-se observar que a sobrecarga é colocada na cauda do animal exigindo que os animais façam mais esforço ao subir. Esse modelo de TF é amplamente utilizado devido sua aplicabilidade e fácil manuseio (DUNCAN *et al.*, 1998; AN *et al.*, 2016; LEITE *et al.*, 2016; TOMAZ *et al.*, 2016), mas é um protocolo que se distancia do modelo praticado por humanos e de difícil controle em relação as variáveis que compõem os programas de prescrição de TF.

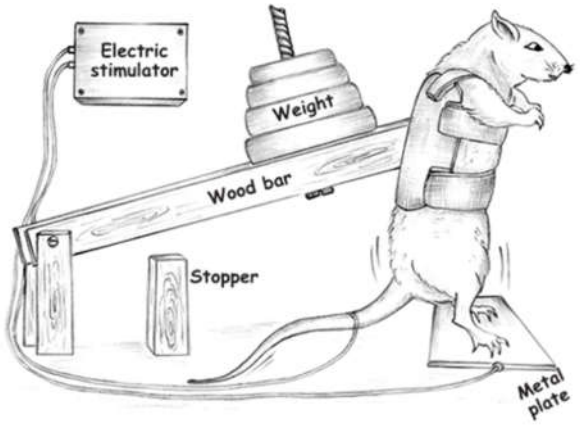

Figura 5 - Modelo de agachamento para ratos com barra de madeira.

O protocolo de agachamento com a utilização de um “braço de madeira” foi desenvolvido em 1992 por Tamaki e colaboradores no Japão (TAMAKI *et al.*, 1992), foi o primeiro a utilizar um aparato um pouco similar ao utilizado por humanos. Muitos estudos experimentais foram desenvolvidos através deste modelo de TF

(BARAUNA *et al.*, 2005; BARAUNA *et al.*, 2007; MELO *et al.*, 2015; NEVES *et al.*, 2016). Barauna e colaboradores demonstraram que o protocolo de agachamento foi capaz de estimular adaptações cardiovasculares impostas pelo TF em animais saudáveis.

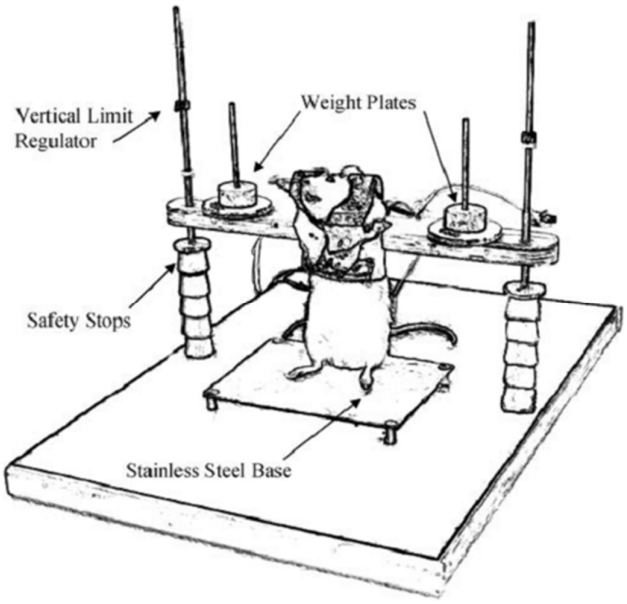

Figura 6 – Modelo de agachamento para ratos com hastes duplas

Na figura 6 é apresentada a ilustração esquemática do protocolo de agachamento criado por um grupo de americanos em 2004 (KRISAN *et al.*, 2004). Esse aparato foi redesenhado a partir do modelo criado por Tamaki e colaboradores (TAMAKI *et al.*, 1992), pois segundo os autores o aparelho com o “braço” era mais propício para repetições com características do treinamento pliométrico. Após o redesenho, o produto final resultou em um aparelho de TF para pequenos animais, que é muito similar ao utilizado por seres humanos. Como podemos observar este aparelho lembra os aparelhos para multi exercícios ou multi força que encontramos em ambientes de treinamento para humanos. Dessa forma, esse foi o protocolo escolhido pelo nosso grupo de pesquisa (Grupo de Pesquisa em Interação Cardiopulmonar - GPIC) para associarmos ao modelo experimental de IM. Entendemos que a escolha de um protocolo de exercício físico para pequenos animais deve possuir características que ajudem a responder algumas perguntas

geradas no ambiente translacional, uma vez que nossos achados são produzidos na ciência básica da bancada para um dia contribuírem como alternativas efetivas no cenário da prática clínica.

O uso do TAC de moderada intensidade na IC tem sido amplamente documentado, tanto na clínica como no modelo experimental, mas a modalidade de TF no cenário das doenças cardiovasculares, especialmente na IC, tem apresentado um número menor de evidências e quando essa modalidade é associada ao TA a quantidade de evidências se reduzem ainda mais. Além disso, o emprego do TF de alta intensidade na IC tem sido pouco observado e quando associado ao TA no modelo experimental de IC, para o nosso conhecimento, não existe até o momento. Dessa forma, hipotetizamos que o TF de alta intensidade associado com o TA de moderada intensidade pode ter efeitos superiores ao TF e TA isolados sobre os parâmetros hemodinâmicos, inflamatórios e de remodelamento cardíaco em ratos com IC. Acreditamos também que o manejo nas variáveis de volume e intensidade do TF pode modular positivamente as respostas da capacidade funcional, através do ganho de força e teste de tolerância máxima ao exercício aeróbico em animais saudáveis e com IC.

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3. Objetivos

3.1 Objetivo geral

Avaliar os efeitos do treinamento de força (TF) de alta intensidade isolado ou combinado com treinamento aeróbio (TA) sobre os parâmetros hemodinâmicos, remodelamento cardíaco e marcadores inflamatórios em ratos com insuficiência cardíaca (IC) induzida pelo infarto do miocárdio (IM).

3.2 Objetivos específicos

Avaliar o impacto de 8 semanas de TF de alta intensidade isolado ou combinado ao TA em animais com IC ou controle sobre:

- a função ventricular esquerda mensurada pelo registro hemodinâmico;
- o remodelamento cardíaco avaliado pela fração de volume de colágeno;
- os marcadores inflamatórios mensurados pela análise da IL-10 e TNF-α;
- o ganho de força máximo avaliado pelo teste 1RM;
- a magnitude do ganho de força mensurado pela diferença percentual entre os testes de 1RM;
- a capacidade funcional mensurada pelo teste de tolerância máxima ao exercício físico (velocidade, tempo e distância percorrida na esteira).

4. Artigo 1

High-intensity resistance training alone or combined with continuous aerobic training improves heart function and collagen, but does not improve inflammatory profile in rats with heart failure

(Artigo formatado pelas normas da revista *Journal of Applied Physiology*, fator de impacto: 3.004)

High-intensity resistance training alone or combined with continuous aerobic training improves heart function and collagen, but does not improve inflammatory profile in rats with heart failure

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Running Head: High-intensity resistance training and aerobic training in HF

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4.1 Abstract

Resistance training (RT) has been associated with positive responses in patients with cardiovascular disease, and when it is combined with continuous aerobic training (CAT), favorable adaptations appear to be even more pronounced. However, the effects of isolated high-intensity RT or those associated with CAT in the case of heart failure (HF) remain unclear.

28 male Wistar rats with HF (90 days old) were allocated to 4 groups: high-intensity RT (RT, n=7), CAT (CAT, n=7), RT and CAT (RT+CAT, n=7) and sedentary (Sed, n=7). Trained animals were subjected to a RT protocol in an adapted squat apparatus for rats (4 bouts, 6-8 reps, 90 s interval, 3x/week, 75% to 85% of one maximum repetition (1RM) for 8 weeks). The animals subjected to CAT performed it 3x/week during 50 min/session at 16 m/min. The animals of the combined exercise regimen performed both the RT and CAT exercise protocols.

The left ventricular end-diastolic pressure (LVEDP), collagen volume fraction and right ventricular hypertrophy were lower in RT, CAT and RT+CAT groups when compared to Sed group ($P<0.05$) for all outcomes. Regarding the inflammatory profile, only the CAT group had greater IL-10 concentrations.

Isolated RT or that combined with CAT was able to improve collagen deposition and attenuate LVEDP. Still, high-intensity RT protocols were not able to alter the inflammatory profile. We concluded that RT combined with CAT improves certain parameters, but it is still necessary to find a better management between intensity and volume of exercise training in the field of cardiovascular diseases.

Keywords: Muscle strength, exercise, hemodynamic function, collagen, acute myocardial infarction.

New & Noteworthy: Our study advances the knowledge of the practices of interventions that utilize the combination of exercises and employ high-intensity in the field of experimental heart failure and our goal was to test if RT with high-intensity in concert with CAT can elicit better responses in hemodynamic function, collagen deposition and the inflammatory status of rats with HF.

4.2 Introduction

Heart failure (HF) is a complex clinical syndrome that results in functional and structural disorders of the heart muscle, consequently yielding symptoms such as dyspnea and fatigue and leading to exercise intolerance in such patients (50). Furthermore, HF is a syndrome characterized by high mortality, frequent hospitalization, reduced quality of life and a demanding complex therapeutic regimen (17). Additionally, the course of the disease results in peripheral changes in skeletal muscles that are closely related to muscle weakness (26) and this scenario produces limitations in heart performance and exercise capacity in these patients (54). Currently, muscle weakness has become a major target of research because the development of skeletal muscle strength is associated with survival of patients with HF (5, 18).

Inflammatory markers have been demonstrated to have an association with the general presentation of HF and can be seen through the existence of multiple biological effects that impact the function of cardiac mechanics (38), pulmonary edema (51) and ventricular remodeling (35). The elevation of pro-inflammatory cytokines has been correlated with reduced mass and muscle strength (53) as well as with functional capacity (44).

Higher levels of interleukins are related to cardiac muscle damage, leading to abnormalities in the extracellular matrix and thereby altering muscle shape, size and ventricular functioning, resulting in a process known as cardiac remodeling (25). Changes in the extracellular matrix of cardiac collagen are connected with impairments in cardiac compliance, valvular function, systolic and diastolic function and in the electrical behavior of the heart (45, 47). Interestingly, physical exercise has produced favorable responses on interstitial collagen fractions in animal models (4, 11, 32, 46) and physical exercise has been described as an effective tool to implement cardiac reverse remodeling in the pathophysiology of HF (1).

Various exercise protocols have been employed as a strong non-pharmacological strategy in animal models of acute myocardial infarction (AMI) (4, 8, 32). The use of resistance training (RT) in the experimental model with healthy rats has exhibited value as a tool for positive adaptations with respect to cardiac function, cardiovascular remodeling (7) and skeletal muscle (43). Recently, rat squatting

models have demonstrated positive responses in diabetes through recovery of cardiovascular function (27) and front to severe impairment of hemodynamic function in experimental models of HF (4).

The effects of aerobic and anaerobic physical training, practiced in combination, require better clarification regarding the type, frequency and intensity of exercise on responses of diastolic function (14). To our knowledge, most studies utilizing animal models of HF have employed the continuous aerobic training (CAT) protocol (11, 32, 33, 46) for observation of the responses of aerobic activity. Therefore, we hypothesized that high-intensity RT combined with aerobic training of moderate intensity may have superior effects to exclusive RT or CAT with respect to strength gain and enhancing functional capacity. The objective of this study was to evaluate whether such responses may be associated with an improvement in hemodynamic function, inflammatory profiles and attenuated collagen deposition in animals with HF.

Glossary

$+dP/dt_{max}$ - Maximum positive Left ventricular change in pressure over time

$-dP/dt_{max}$ - Maximum negative Left ventricular change in pressure over time

1RM - Test of one repetition maximum

AMI - Acute myocardial infarction

CAT - Continuous aerobic training

CVF - Collagen volume fraction

DBP - Diastolic blood pressure

ETT - Exercise test tolerance

HF - Heart Failure

LV - Left ventricle

LVEDP - Left ventricular end-diastolic pressure

LVSP - Left ventricular systolic pressure

LVW/BW - Left ventricular weight-to-body weight

MAP - Mean arterial pressure

MW/BW - Myocardial Weight-to-body weight

PC - Pulmonary congestion

RT - Resistance training

RV - Right ventricle

RVW/BW - Right ventricular weight-to-body weight

SBP - Systolic blood pressure

TNF-α - Tumor necrosis factor – alpha

VHR - Ventricular heart rate

4.3 METHODS

This experimental study was based on ARRIVE (Animals in Research: Reporting In Vivo Experiments) guidelines (20).

Sample size, animals and ethical approval

To calculate the sample size, we used the relative values of maximum strength gain obtained from a previous study in our laboratory with infarcted rats (4). To detect the minimum difference of 1.40 (international units) with an expected residual standard deviation of 0.75, including power of 80% and an α error of 5%, for a total of 4 groups, the final number for each experimental group was 8 animals. Considering an expected mortality between 35-40% (9, 19) during the surgical induction of AMI, the final sample size was 13 animals per group. Therefore, 52 male Wistar rats (220 to 270g, 90 days of age) were obtained from the breeding animal unit of the Universidade Federal de Ciências da Saúde de Porto Alegre and were housed under standard conditions (food and water *ad libitum*, a light and dark cycle of 12:12 hours and 22 ° C ambient temperature). This investigation followed the rules established by the guide for care and use of animals in experiments published by National Institute of Health (NIH publication no. 41-87, revised in 2011). All the procedures defined in this study were approved by the Ethics Committee in Research of Universidade Federal de Ciências da Saúde de Porto Alegre (protocol 199-13).

Acute myocardial infarction induction

The animals were weighed and anesthetized by inhalation with 2% isofluorane (Isofluorine, 100 ml - Cristália) in 100% oxygen (28) in a clear glass bell jar and were immediately artificially ventilated afterwards (Samway VR 15, 60 breaths / minute and a fraction of 100% oxygen inspired). Following thoracotomy, the ligation of the left descending coronary artery was performed to induce AMI as described previously (9, 19, 32).

Experimental design

Following AMI, 28 animals were kept for a period of five weeks post-surgically (time needed to reach the development of a HF status) (15, 37). The animals were allocated into 4 groups: sedentary HF (Sed), resistance training HF (RT), continuous

aerobic training HF (CAT) and resistance training combined with continuous aerobic training HF (RT+ CAT). Figure 1 depicts the timeline in relation to the experimental procedures.

Resistance Training

Adaptation

Five weeks after performing surgery, the animals of the AMI training groups were subjected to a familiarization period with the adapted squat apparatus (22). Specifically, the animals were placed in the apparatus and encouraged to perform 5 to 10 repetitions with 40% to 60% of body weight for one week. This charge is considered low intensity given that other studies have reported untrained rats can lift up to three times their body weight upon first contact with the squat apparatus (4, 42).

1RM test

To determine training load, all animals were subjected to the 1RM test. The 1RM test has been widely used in experimental designs of resistance training (7, 22, 42). The test would be validated if the maximum load to be lifted once (resistance break) in the squat apparatus was in fact lifted, and the total amount recorded in the test represented the total load (100%). From the values obtained, the percentage load for performing the training protocol was determined. However, in response to the RT protocol, the strength gains became evident and new tests were performed every two weeks to adjust the training load.

RT protocol

The animals were placed in a neoprene vest (polychloroprene synthetic elastomer) and then left in the standard suspension position where they remained upright on their lower limbs. An electric stimulus (4-5 mA, 1 s duration, with a 3 s interval between each repetition) was applied to the rats' tails through an adapted surface electrode (4). As a result, flexions and extensions occurred in the lower limbs of the animals and there was subsequent lifting of the load imposed on the squat

device. These parameters were adopted in order to allow long-term application without the risk of tissue damage (43). The training protocol lasted for 8 weeks with 4 bouts of 6 to 8 repetitions with 90 s rest periods between each series three times per week with 75% load and 85% of the 1RM test, method adapted from Baraúna et al. (7). Therefore, the animals were exercised with 75% of 1RM in the first week and 85% of 1RM during the second week of training, after the end of every two weeks of training the 1RM test was performed to recalculate the load and verify the strength gain. The intensities of this protocol were classified as vigorous at near-maximum to maximum (high-intensity) according to the guidelines of the American College of Sports Medicine (ACSM) (16). The training program follows the recommendations of the American Physiological Society (APS) (21).

CAT

Adaptation treadmill

Five weeks after AMI induction surgery, the animals of the training groups were subjected to a period of adaptation to the treadmill. The rats were placed on the treadmill (0% inclination) and encouraged to perform a running exercise at a speed of 10m / min for five minutes on the first and second day and ten minutes on the third, fourth and fifth days.

Exercise test tolerance / incremental load test

The animals were subjected to an exercise tolerance test (ETT) five weeks after AMI surgery and after eight weeks of training. The animals were adapted to an ergometer for 5 days (10 minutes / day) before the tests. The protocol employed consisted of a run on the treadmill with an inclination of 15 ° until exhaustion. The protocol began with a speed of 5 m / minute and there was an increment of 5 m / minute every 3 minutes until exhaustion of the animal (13).

Aerobic training protocol

The training protocol lasted for eight weeks, with 50 minute runs 3 times per week at one 10m / minute speed, which was progressively increased up to 15m / minute (0% inclination) over the proceeding weeks until the end of training. The

training load was applied to 60% of the maximum speed obtained in the maximum tolerance treadmill test. The training program followed the recommendations of the American Physiological Society (APS) (21).

Protocol of combined physical training

The Animals of the RT group combined with CAT initially performed the RT and immediately at the end of training, the animals were subjected to CAT.

Evaluation of Hemodynamic Function

Surgical preparation and hemodynamic evaluation

The recordings were performed 48 hours after the end of the training protocol. The animals were anesthetized with Ketamine (80 mg/kg, i.p.) + Xylazine (12 mg / kg, i.p.) and intubated (Gelko - 14G). A polyethylene catheter model P50 was introduced into the left ventricle (LV) via the right carotid artery. Blood pressure was recorded for 5 minutes immediately following the catheter being positioned within the LV to perform the registration of the ventricular pressure. Data were recorded through a pressure transducer (strain-gauge, Narco Biosystem Miniature Pulse Transducer RP-155, Houston, Texas, USA) coupled to a pressure amplifier (Stoelting, wood dated, Illinois, USA). The analog pressure signals were digitized by the data acquisition system (CODAS-Data Acquisition System, Akron, OH, USA) with a sampling rate of 2.000 Hz. This data was then used to determine the diastolic blood pressure (DBP), systolic blood pressure (SBP), mean arterial pressure (MAP), heart rate (HR), left ventricular systolic pressure (LVSP), end-diastolic pressure of the left ventricle (LVEDP) and maximum positive left ventricular change in pressure over time ($+dP/dt_{max}$) as well as maximum negative left ventricular change in pressure over time ($-dP/dt_{max}$) (4).

Blood sample and collection of tissue

Blood collection was conducted with the tube already positioned in the left carotid artery and samples were taken in two 1.5 ml tubes containing 3.2% sodium citrate (1: 9 v / v) and next centrifuged at 500 x g at 4 ° C. The plasma was stored at -22 ° C. Immediately after the hemodynamic assessment and blood collection, the

animals were sacrificed by decapitation and the heart, lung and liver tissues were harvested (4).

Cardiac hypertrophy, pulmonary and hepatic congestion

The rat hearts were removed and weighed without blood in the chamber and without the atriums. The LV and right ventricle (RV) were dissected from each heart, separated and weighed. For the determination of cardiac hypertrophy, the organs were expressed as a ratio to body weight (tissue weight / body weight - mg/g) (4, 12, 32). To determine lung and liver congestion, the liver and lungs of each animal were removed, weighed and subsequently dried (80 ° C) for 48 hours and weighed again to determine the volume of water. The proportions of wet and dry weight of tissue were used to establish the percentage of water in the tissues as an indication of pulmonary congestion (PC) and hepatic congestion. To evaluate congestion, the following formula was utilized:

$$Congestion\ (\%H_2O) = \frac{(Wet\ weig\ \ t\quad Dry\ weig\ \ t)}{(Wet\ weig\ \ t\ x\ 100)}$$

Determining the size of the infarcted area

Directly following the calculation of the weight of the heart, the left ventricles were filled with a latex pad and submerged in buffered formaldehyde for 10% over 24 h for subsequent analysis of the size of the infarcted area. The size of the infarcted area was determined by histological evaluation adapted from the method previously described by Martinez et al. (23). The measurements were performed in the mid-ventricular slices (5-6 mm from the tip) under the assumption that cuts from that point exhibited a close linear relationship with the sum of the measures of all heart cuts (34). Therefore, 6 mm thick of medium-ventricular cut sections were stained with Masson's trichrome. The ImageJ software version 1:49 was used to obtain the length of the whole circumference of the endocardium and the endocardium tracking the length of the infarcted region formed from two medium-ventricular sections of the LV via manual planimetry. The fraction of the infarcted area of the LV was calculated from these measurements. All measurements of the infarct areas were by a single technician who was blinded in relation to the hemodynamic data and work of the group.

Analysis of interstitial collagen deposition in the heart muscle

Cross cuts were performed on the LV and these fragments were fixed in 10% buffered formaldehyde for subsequent inclusion in paraffin, a method adapted previously (24, 48).

Histological sections of 6 microns were stained with picrosirius Red (PSR) and were employed for measurements of CVF present in the interstices of LV cardiac tissue. The readings were performed using polarized light (48) with an Olympus microscope attached to a video camera that sent a digital image to a computer with the image analysis program, Image Pro-plus (Media Cyberetics, Silver Spring, Maryland, USA). It was analyzed at 40 fields per ventricle with a 40X objective. The fields selected were located far from the infarcted area and distant from the pericardial region as previously described (4). The CVF was defined as the sum of all areas of interstitial collagen tissue (highlighted by polarization) divided by total tissue area (24).

Determination of plasmatic levels of interleukins

After hemodynamic recording, blood collection was performed to determine the plasma concentrations of TNF-α and IL-10. The markers were assessed through a Milliplex Map kit (RCYTO-80K, Millipore - Billerica, MA, USA) for cytokine rats. Milliplex Map is based on the Luminex xMAP technology following the manufacturer's recommendations. These techniques have already been conducted in other experiments in our laboratory (4, 33). All cytokines are presented in pg/ml.

Statistical analysis

Each variable and group were expressed as mean ± SD. The Shapiro-Wilk test was carried out to evaluate the normality of all variables. We used one-way analysis of variance (ANOVA) followed by a Tukey's post hoc test to compare variables between groups (body weight, infarcted area size, cardiac hypertrophy, pulmonary and hepatic congestion, hemodynamic variables, maximum percentage gain strength, interleukin-10 and TNF-α and fraction of collagen content). A Two-way repeated measures ANOVA with subsequent Tukey's post hoc test was employed to compare the strength gain between groups at different experimental times and also to compare speed, distance and time in the ETT. Pearson's correlation test was applied to examine the relationship among gain maximum strength and

hemodynamic variables, cardiac hypertrophy, pulmonary congestion, collagen volume fraction, interleukin-10 and TNF-α and speed, time and distance in the ETT. P values <0.05 were considered significant. Statistical and construction of graphical analysis was with GraphPad Prism version 6.01 (GraphPad Software, San Diego, CA, USA) for Windows. Sigmaplot version 12.0 (Systat Software Inc., San Jose, CA, USA) software was utilized for complementary statistical analysis.

4.4 RESULTS

Mortality, exclusion and adverse effects

The mortality with AMI induction surgery was ~ 39% (20/52). Only those animals that completed the experiment were included in the statistical analysis. The study only consisted of animals with a large infarct area (≥ 40%). Therefore, we did not obtain the hemodynamic record of two animals, a member of the RT+CAT group (1) and a member of the Sed group (1). As well, one animal did not reach 75% during the aerobic training protocol because of stress from hyperventilation (member of CAT), and one animal did not complete 75% of the strength training protocol because they lost sensitivity to electrical stimulation (RT group member). As could be expected, these animals were excluded from the study. Following exclusion of animals, all experimental groups were divided as follows: Sed (n=7), RT (n=7), CAT (n=7), and RT+CAT (n=7). No deaths occurred during the exercise protocols.

Morphological variables and congestion in organs

All data are summarized in Table 1. The initial and final body weights of the animals were similar between groups in the pre- and post-training states, as were the sizes of the infarcted areas, suggesting that all groups developed a similar syndrome of HF. We observed that the Sed-IC group showed greater hypertrophy when compared to the group that received the combined training. These data demonstrate that RT when combined with the CAT may attenuate right ventricular hypertrophy (RVW/BW) in rats with HF syndrome, all forms of training (RT, CAT and RT + CAT) prevent the increase in PC (%water) that took place in the Sed group.

Hemodynamic evaluation

All data are summarized in Table 2. No differences were observed in the following variables: heart rate (HR), SBP, DBP, MAP and LVSP between groups. The groups subjected to RT protocols demonstrated attenuation in LVEDP compared to the Sed. These findings indicate that physical training was able to induce favorable responses on left ventricular dysfunction by lowering LVEDP and the RT mode brought about more pronounced responses in this marker of cardiac dysfunction when compared to the CAT. Moreover, the Sed group had LVEDP values above 20 mmHg, suggesting the presence of severe left ventricular dysfunction compared to the other groups. Interestingly, the CAT group had a reduction in ventricular heart rate (VHR) compared to group Sed, and there was also observed increased values in the $+dP/dt_{max}$, mmHg/s and $-dP/dt_{max}$, mmHg/s of the CAT group compared to the Sed group. The RT+CAT group exhibited an increase in $-dP/dt_{max}$, mmHg/s values versus the Sed group.. In addition, improvements were seen in the CAT group with respect to hemodynamic variables, like VHR and, $+dP/dt_{max}$, mmHg/s and $-dP/dt_{max}$, mmHg/s which represent cardiac contractility and relaxation, respectively. The combined training protocol was also able to enhance cardiac relaxation as reflected by an increase of $-dP/dt_{max}$, mmHg/s.

Maximum Strength Gains: Responses Intra- and Extra-Groups

Effects within groups

Figure 2 details the gain of absolute force (A) and relative force (B) of the experimental groups in the segment over time (1st 1RM, 2nd 1RM, 3rd 1RM and 4th 1RM). In terms of the 1st 1RM, there were no differences between groups in absolute and relative tests, suggesting that all groups started the protocol under similar conditions. The RT and RT+CAT groups presented gain maximum strength, both in absolute and in the relative load in all tests compared to the initial test (1st 1RM vs 2nd 1RM, 3rd 1RM and 4th 1RM). Of note is the fact that the CAT group showed an increase in absolute load when compared to the 2nd 1RM versus the 1st 1RM and the Sed group demonstrated a rise in absolute load compared to the 3rd 1RM versus the 1st 1RM. However, when the load is related to their body weight, the CAT and Sed group had no strength gains and it was still possible to observe that the CAT group showed a reduction in strength gains when comparing the 4th 1RM with 1st 1RM.

Effects between the groups

Figures 2A and 2B depict the absolute and relative strength gains between the groups. When reviewing the absolute strength gains, we can observe that the groups that were subjected to the RT protocol alone or combined with CAT had an increase in their average values in the 2nd 1RM, 3rd 1RM and 4th 1RM compared to the Sed and CAT groups. Only the relative load of the 2nd 1RM differences were not observed between the RT+CAT group compared to the CAT group. For the 3rd 1RM and 4th 1RM, the differences remained.

Magnitude strength gain between the percentage differences of maximum load tests

Figures 2C and 2D portray the strength gain percentages between the tests of the experimental groups undergoing the isolated RT protocol, RT, or combined with CAT, RT+CAT. In Figure 2C, strength gains in the absolute load by the percentage difference between the 1st and 2nd RM for the RT and RT+CAT groups with percentages of 26.1% and 22.3% are seen, respectively. The percentage difference between the 1st and 2nd RM tests was higher when compared with the percentage differences of the subsequent tests (2nd 1RM vs 3rd 1RM and 3rd 1RM vs 4th 1RM). Figure 2C and 2D also show the observed changes in the percentage of absolute and relative strength gains of the RT and RT+CAT groups from the percentage differences between the 1st 1RM vs 2nd 1RM tests and these values are different when compared to the percentage differences of the 2nd 1RM vs 3rd 1RM tests. Interestingly, the percentage differences in the magnitude of strength gains between the 2nd 1RM vs the 3rd 1RM and the 3rd 1RM vs the 4th 1RM tests showed no difference both in absolute load as well as the relative load to body weight. These results suggest that during the protocol, there would be maximum strength gains, though on a smaller scale when compared to the effects of the initial two weeks of the RT protocol.

ETT

Figures 3A, 3B and 3C exhibit the variables analyzed in the ETT of the pre- and post- intervention experimental groups. In the pre-ETT intervention, there were no differences between the groups, suggesting that all groups presented similar

conditions before the start of interventions. Regarding post-ETT (Fig. 3), the groups subjected to CAT protocols demonstrated an increase in speed, time and distance compared to pre-ETT. In the comparison between groups, it was also observed there were differences between endurance-trained animals when compared with the Sed and RT groups. Additionally, no differences were found in the pre- and post-test between groups trained with the CAT protocol. These results demonstrate that CAT, performed alone or accompanied by RT, positively interfere with the capacity of animals with HF to achieve higher speeds, quicker times and longer distances, but these effects are not enhanced by combination with RT.

Inflammatory profile

In Figures 4A and 4B are shown the values for IL-10 and TNF-α of the experimental groups. The group subjected to aerobic training had higher IL-10 levels compared to the Sed group and there was no difference compared to RT and RT+CAT. No differences were observed between the groups for the pro-inflammatory marker TNF-α after the intervention protocols. The TNF-α/IL-10 ratio was also measured and no differences were determined between groups. This suggests that an aerobic training protocol of moderate intensity can positively modulate IL-10 but not change the pro-inflammatory response of TNF-α.

Interstitial collagen volume fraction

The Figure 5 shows, the values of interstitial collagen volume fractions of the heart in the experimental groups after 8 weeks of an exercise protocol are shown. The groups that were subjected to the RT isolated protocol or combined CAT exhibited reduced volumes of interstitial collagen deposition when compared with the Sed group.

Correlations between central responses (hemodynamics and collagen volume fraction) and maximum strength gain between the experimental groups

Figures 6A, 6B, 6C, 6D and 6E portray correlations between gain strength and the hemodynamic parameters and interstitial collagen fraction. We observed a significant correlation between LVEDP and the 4th 1RM, represented by the ratio of 4th 1RM/BW (Figure 6A) and between LVEDP and the change in maximum strength

gain (Figure 6B). It was also observed that there was a significant correlation between LVEDP and CVF (Figure 6C). In addition, it was observed that there were significant correlations between CVF and the maximum strength gain as well as the change in maximum power gain (Figure 6D and Figure 6E, respectively). Overall, the correlations demonstrated that the gain in maximum strength expressed by the last 1RM test and change in strength gain are closely related to the reduction of LVEDP and with CVF.

4.5 DISCUSSION

To our knowledge, this is the first study to investigate the effects of 8 weeks of high-intensity RT (75% - 85% of 1RM) and CAT of moderate intensity (60% of the speed of ETT) both practiced alone or in combination in an experimental rat model with HF. Here, we demonstrated that the groups subjected to the RT protocol alone or combined with CAT responded positively with strength increase when observing 1RM tests in absolute load (g) and relative (1RM/BW) and were also observed in exercise tolerance obtained through ETT in animals subjected to the aerobic training protocol alone or combined with RT. Based on the results presented in this work, the groups that were exposed to exclusive or combined exercise models showed improvement in the following variables: strength gain, LVEDP, $+dP/dt_{max}$, $-dP/dt_{max}$, PC (% water), right ventricular hypertrophy, IL-10 and CVF. The maximum strength gain found in our study is an important finding as many studies have shown that muscle weakness is an independent risk factor for mortality in populations with HF (5, 18). In addition, the structural modification of skeletal muscle, which is related to muscle weakness, became the target of research in various scenarios of chronic diseases (10).

Coronary artery ligation produces a marked ventricular dysfunction that is closely related to the infarct area size (4, 19, 32) and therefore may be observed as a major impairment in hemodynamic function. In the present study, the infarcted areas were close to ~43%, which reflects directly a high LVEDP. Among the hemodynamic responses, we are able to highlight LVEDP, an important marker of ventricular dysfunction in HF (29), and, in this study, we observed an attenuation of this variable in the RT group (67.8%), CAT (48.7%) and RT+CAT group (71.1%) based on the LVEDP Sed group. We also saw that those animals that had an increase in muscular

strength showed lower LVEDP values as demonstrated by the negative correlations in Figures 6A and 6B (r = -0.62 and r = - 0.73, respectively). Previous studies in our laboratory with the HF experimental model associated with respiratory muscle training (19), swimming (33) and CAT (32) showed similar responses in LVEDP, but the effects are more pronounced with the RT protocol alone or combined with CAT as in this study. However, in an experimental study with infarcted rats subjected to a RT protocol of moderate to high-intensity (65% -75% of the 1RM test), we found a greater number of positive responses, e.g., attenuation of LVEDP, an increase in the LVSP and in the $+dP/dt_{max}$ and $-dP/dt_{max}$ (4).

The $+dP/dt_{max}$ and $-dP/dt_{max}$ exhibited favorable changes only in the CAT group because the group with combined exercise showed improvement solely in $-dP/dt_{max}$. Results similar to our CAT group have been reported in experimental studies of HF with various forms of interventions (19, 32, 33) as well as in ovariectomized rats subjected to AMI and exposed to CAT (2).
Therefore, we can point to the intensity of the training protocol, independently of the type of exercise, as the main factor responsible for stimulating beneficial responses in hemodynamic variables because protocols with moderate intensity, in both the RT (4) and CAT (31), provided positive responses in a larger number of hemodynamic variables. On the other hand, from a translational perspective, clinical studies have suggested there are controversial effects from the impact of exercise on systolic and diastolic function after acute myocardial infarction (3, 14, 40).

Lung congestion is a strong negative component of the HF syndrome both in clinical (36) as well as in experimental models of HF (15, 29). The physical exercise protocols performed alone or in combination, in the present study, were able to lower pulmonary congestion as a consequence of HF syndrome and these responses were similar to findings in other studies that employed RT or CAT as the primary intervention in rats with HF (4, 11, 31). Notably, a study using the CAT protocol associated with Caverdiol in rats with HF also showed a reduction in PC, demonstrating that the combination of interventions can be a interesting strategy. Furthermore, another experimental study demonstrated a significant reduction in PC of ovariectomized rats with AMI that performed CAT when compared to a sedentary group with AMI (2). This indicates that aerobic exercise training is an effective

strategy to positively attenuate change in the accumulation of water in the pulmonary system.

Additionally, we observed in our study a reduction in the ratio of right ventricle weight-to-body weight (RVW/BW) in animals of the RT+CAT group. Such a ratio between heart tissue weight and body weight has been extensively employed as a marker of cardiac hypertrophy (12, 41). This outcome was also found in a previous study that utilized only the RT isolated in rats with heart failure (4). In another study conducted by Jaenisch et al. (19) utilizing ventilatory muscle training, It was also demonstrated that there was a reduction in right ventricular hypertrophy in rats with heart failure, though other findings in experimental model involving mice and rats were not observed in terms of change in the right ventricle after the interventions proposed (2, 11). Perhaps there are differences in experimental models of HF (mice vs rat), the method of inducing of HF, size of the infarct area and characteristics of interventions exist between studies and yield varying results.

Elevated levels of pro-inflammatory cytokines are associated with a poor prognosis and increased severity of HF (6). Moreover, the imbalance of inflammatory markers are also connected to increased mortality in patients with HF (39). On the other hand, physical exercise has been shown to be a potent non-pharmacological strategy to induce improvements on the inflammatory profile front in the scenario of HF in experimental (4, 8, 33) as well as clinical (30) models. In the present study, IL-10, TNF-α and the ratio of IL10/TNF-α were evaluated in the groups that were subjected to high-intensity RT or CAT, both in isolated and combined mode, in the present study, we observed an increase in plasma levels of IL-10 only in the CAT group, showing no changes in plasma levels of these inflammatory markers for the other groups.

Interestingly, the combined group did not show improvement in this variable, possibly because the total volume of training may have interfered negatively to increased plasma levels of IL-10. However, certain studies with experimental models of HF also utilizing CAT or swimming demonstrated increases in IL-10 levels and the ratio of IL-10/TNF-α (8, 31, 33) and a reduction in plasma levels of TNF-α (31). These responses may be associated with the type of exercise intensity of the protocols for the studies mentioned as the intensity percentage did not exceed 65%. Additionally, we demonstrated that the RT protocol with lower intensity compared to the present

study (65%-75% vs 75%-85%) was able to positively modulate serum levels of IL-10 and IL-6 as well as the IL-10 / TNF-α ratio (4).

The modification of extracellular matrix collagen is a natural process part of an adaptive response to increased mechanical load on the heart during exercise, but the chronic overload arising from cardiac injury develops a negative response with respect to distensibility of the heart, valvular function, systolic and diastolic dysfunction and electrical transmission, ultimately leading to myocardial arrhythmia in HF (45, 47, 52). In the present study, the interstitial collagen accumulation of animals in the Sed group was higher when compared to groups subjected to physical training, demonstrating a possible reverse remodeling of the extracellular matrix. These results are similar to the responses observed with the CAT protocol performed by Xu et al. (49) and Nunes et al. (32). Recent investigations have also shown similar responses with combined protocols of aerobic training and supplementation use based on leucine (11) in addition to the use of Caverdiol medication (46). Here, a positive correlation was also seen between increased collagen accumulation and LVEDP (r= 0.64; *P*= 0.0002, Figure 6C), and this response helps to understand the relationship between hemodynamic injury and structural changes of the heart. Yet, it was observed in this study that an increase in muscle strength can influence the reduction of the accumulation of collagen, and these responses are demonstrated by the correlation of Figures 6D and 6E (r= -0,49 and r= -0,57; P= 0.007 for both, respectively). Additionally, the reduction in collagen volume was also found after application of RT protocol for rats with HF, but the intensity did not exceed 75% of the RM test (4).

These are some limitations of this study. First, the lack of robust analyzes in muscle tissue could help better understand the possible adjustments to the high-intensity training in the MI model, especially the balance between the molecular pathways of anabolism and catabolism. Second, the evaluation of oxidative stress and DNA damage in the gastrocnemius, soleus, lung and heart could explain the damage caused by the experimental model of acute myocardial infarction and the possible effects of exercise protocols in these structures. Third, the absence of a load cell coupled to the squat apparatus for rats could help to increase the precision of maximum strength tests and control of the loads during the training protocol.

CONCLUSIONS

The RT protocol of high-intensity, performed alone or combined with CAT of moderate intensity, was able to increase the strength in animals with HF syndrome and this response is closely related to the improvement of ventricular function. This positive outcome was represented by attenuation of LVEDP and through the reduction of interstitial collagen deposition, demonstrating that the strength gain stimulates favorable central responses during the advancement of HF. Nevertheless, the high-intensity RT protocol does not appear to offer benefits in terms of inflammatory markers (IL-10 and TNF-α) - just the CAT protocol of moderate intensity was capable of exhibiting a positive influence on IL-10. We emphasize that the exercise protocols should be very well balanced in relation to the volume, intensity and frequency as they these variables can directly affect the outcome of physical training programs. We recommend that future experimental studies investigate the influence of exposure time and both exercise protocols in isolation as well the protocols combined in the field of cardiovascular diseases, especially in the complex case of HF syndrome.

Acknowledgements

We are grateful to Prof. Luís Fernando Deresz, Terezinha Stein, Rosalva Theresa Meurer, Ignês Cristiane de Souza Paiva and Mário Pereira (in memoriam) for support during this study.

Funding sources

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil.

Authors' contributions

Design and development: JPA, RBN, DCF, RBJ, GPS e PD. Development of the experiments: JPA, RBN, DCF, RBJ, GPS e PD. Data analysis: JPA, RBN, DCF, RBJ, GPS e PD. Contribution with reagents / materials / analysis tools: JPA, RBN, DCF, RBJ, GPS e PD. Article Writing: JPA, RBN, DCF, RBJ, GPS e PD.

Conflicts of interest

The authors declare that they have no conflict of interest.

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4.7 LEGENDS TO THE FIGURES

Figure 1. Timeline of experimental procedures (study design). Acute myocardial infarction, AMI; resistance training, RT; continuous aerobic training, CAT; exercise tolerance test, ETT; 1st test of a maximum repetition, 1st 1RM; 2nd test of a maximum repetition, 2nd 1RM; 3rd test of a maximum repetition, 3rd 1RM; and 4th test of a maximum repetition, 4th 1RM.

Figure 2. **A**: Maximum strength gain over time, absolute load. **B**: Maximum strength gain over time, relative load. **C:** Magnitude of absolute strength gain between the segments of the tests (1st 1RM vs 2nd 1RM; 2nd 1RM vs 3rd 1RM; and 3rd 1RM vs 4th 1RM). **D:** Magnitude of the relative strength gains between the segments of the tests (1st 1RM vs 2nd 1RM; 2nd 1RM vs 3rd 1RM; and 3rd 1RM vs 4th 1RM). Values are mean ± SD, n= 7 for all groups. Sedentary group with HF, Sed; resistance training group with HF, RT; continuous aerobic training group with HF, CAT; resistance training combined with continuous aerobic training group with HF, RT+CAT. Graph **A** and **B**, intragroup analysis: *P<0.01 RT and RT+CAT groups comparing the 1st 1RM test with absolute and relative load. †P<0.05 - Sed and CAT groups compared to the 1st 1RM test with absolute load. ‡P<0.05 CAT group compared to the 4th MR test with 1st 1RM test with relative load. Analysis among groups: #P<0.01 RT and RT+CAT groups compared to the 2nd, 3rd and 4th 1RM test of the Sed and CAT with absolute and relative load. Graph **C** and **D**: *P<0.05 compared the percentage differences of the 2nd 1RM vs 3rd 1RM tests and the 3rd 1RM vs 4th 1RM tests for the RT group. †P<0.05 compared the percentage differences of the 2nd 1RM vs 3rd 1RM tests for the RT+CAT group.

Figure 3. Exercise tolerance test (ETT). **A**: speed (m/s); **B**: Time (s) and **C**: Distance (m). Values are means ± SD, n= 7 for all groups. The sedentary group with HF, Sed; resistance training group with HF, RT; continuous aerobic training group with HF, CAT; resistance training combined with continuous aerobic training group with HF, RT+CAT. intragroup analysis: *P<0.001; CAT and RT+CAT groups compared to the pre-intervention time. Analysis between groups: †P<0.001 compared to groups Sed and RT.

Figure 4. Analysis of inflammatory markers in plasma. **A**: IL-10 e **B**: TNF-α. Values are mean ±SD. Sedentary group with HF (Sed, n= 6); resistance training group with HF (RT, n= 6); continuous aerobic training group with HF (CAT, n=5); resistance training combined with continuous aerobic training group with HF (RT+CAT, n= 5). *P< 0.05 compared to the Sed group.

Figure 5. Collagen deposition analysis expressed by the percentage fraction of interstitial collagen volume. The arrows in the images indicate collagen detected through quantification of polarized light. Values are mean ±SD, n= 7 for all groups. **A:** sedentary group with HF, Sed; **B:** resistance training group with HF, RT; **C:** continuous aerobic training group with HF, CAT; **D:** resistance training combined with continuous aerobic training group with HF, RT+CAT. **E:** representative graph of the quantification of collagen volume fraction. *P< 0.05 compared to Sed group.

Figure 6. Correlations between the left ventricle end-diastolic pressure (LVEDP) with maximum gain in strength and volume fraction of collagen. red circles (●), Sed; dark square (■), RT; dark triangle (▲), CAT; and blue triangle (▼), RT+CAT. **A:** LVEDP with 4th 1RM/BW; **B:** LVEDP with Δ maximum strength gains (%); **C:** LVEDP with fraction of collagen volume (%); **D:** fraction of collagen volume with 4th 1RM/BW; **E** fraction of collagen volume with Δ maximum strength gains (%). Statistical analysis: Pearson's correlation test.

Table 1. Body weight, infarcted area size, cardiac hypertrophy, lung and liver congestion.

	Sed	RT	CAT	RT+CAT	*P One way ANOVA*
Initial weight, g	264.3±9.1	262.6±20.4	270.4±9.5	274.3±7.8	0.401
Final weight, g	359.7±41.6	344.6±34.06	350.0±26.81	370.8±21.5	0.523
Infarcted area%	42.52±2.58	41.51±1.77	42.82±2.21	44.63±3.34	0.169
MW/BW mg/g	3.87±0.55	3.72±0.42	3.60±0.18	3.39±0.35	0.827
LVW/BW mg/g	2.71±0.48	2.96±0.32	2.73±0.30	3.11±0.52	0.274
RVW/BW mg/g	1.16±0.39*	0.76±0.18	0.86±0.25	0.61±0.48	0.030
Pulmonary congestion (PC) %	78.19±2.78†	70.17±4.06	72.52±2.47	73.00±1.46	<0.001
Hepatic congestion (HC) %	69.87±0.53	70.28±0.80	71.09±2.28	70.10±0.73	0.743

Values are means ± SD; n= 7 for all groups. All groups with HF. Sedentary group, Sed; resistance training group, RT; continuous aerobic training group, CAT; resistance training combined with continuous aerobic training group, RT+CAT. Ratio of the total myocardial Weight to body weight, MW/BW; ratio of left ventricular weight to body weight, LVW/BW e ratio of right ventricle weight to body weight, RVW/BW. *compared to RT+CAT and †compared to all groups.

Table 2. Hemodynamic variables of the experimental groups.

	Sed	RT	CAT	RT+CAT	*P One way ANOVA*
HR, bpm	249.4±40.6	269.3±52.6	253.4±71.0	244.7±57.7	0.790
SBP, mmHg	92.8±15.5	107.4±16.6	110.6±27.6	114.1±15.39	0.237
DBP, mmHg	76.0±12.5	83.05±10.2	82.1±11.7	92.9±12.5	0.186
MAP, mmHg	84.6±13.3	97.1±16.2	98.4±22.1	103.6±12.8	0.203
LVEDP, mmHg	25.6±4.1*	8.12±2.8	13.1±2.3†	7.4±3.1	<0.001
LVSP, mmHg	88.5±21.1	102.6±10.2	113.0±29.8	106.6±3.9	0.117
VHR, bpm	246.9±39.1	236.3±33.6	210.2±9.9‡	226.4±13.1	0.039
$+dP/dt_{max}$, mmHg/s	3553.3±1064.0	4921.6±782.7	5463.4±1938.0‡	4929.4±449.5	0.035
$-dP/dt_{max}$, mmHg/s	-2260.0±597.1	-3279.0±560.4	-3759.3±1625.0‡	-3293.1±247.1‡	0.012

Values are means ± SD; n= 7 for all groups. All groups with HF. Sedentary group, Sed; resistance training group, RT; continuous aerobic training group, CAT; resistance training combined with continuous aerobic training group, RT+CAT. Heart rate, HR; Systolic blood pressure; SBP; diastolic blood pressure, DBP; Mean arterial pressure, MAP; end diastolic pressure of the left ventricle, LVEDP; left ventricular systolic pressure, LVSP; ventricular heart rate, VHR; maximum positive left ventricular change in pressure over time, $+dP/dt_{max}$; Maximum negative left ventricular change in pressure over time, $-dP/dt_{max}$. *compared to all groups. †compared to RT and RT+CAT. ‡compared to Sed.

Figure 1.

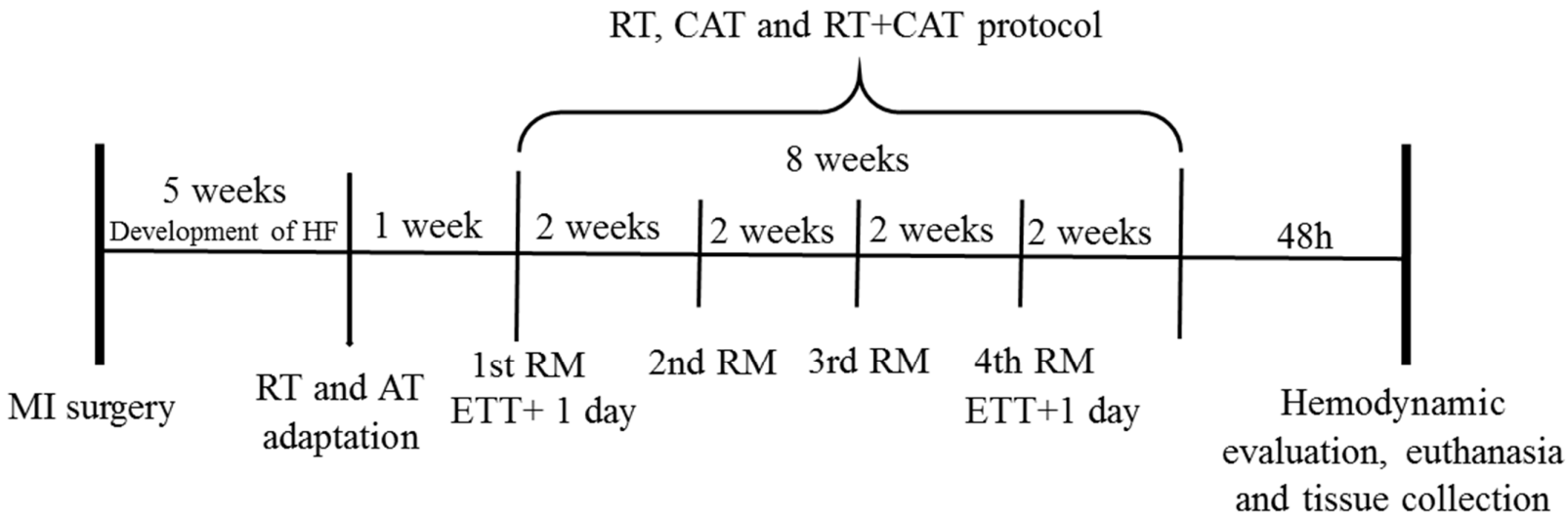

RT, CAT and RT+CAT protocol
8 weeks
5 weeks
Development of HF
1 week
2 weeks
2 weeks
2 weeks
2 weeks
48h
MI surgery
RT and AT adaptation
1st RM ETT+ 1 day
2nd RM
3rd RM
4th RM ETT+1 day
Hemodynamic evaluation, euthanasia and tissue collection

Figure 2.

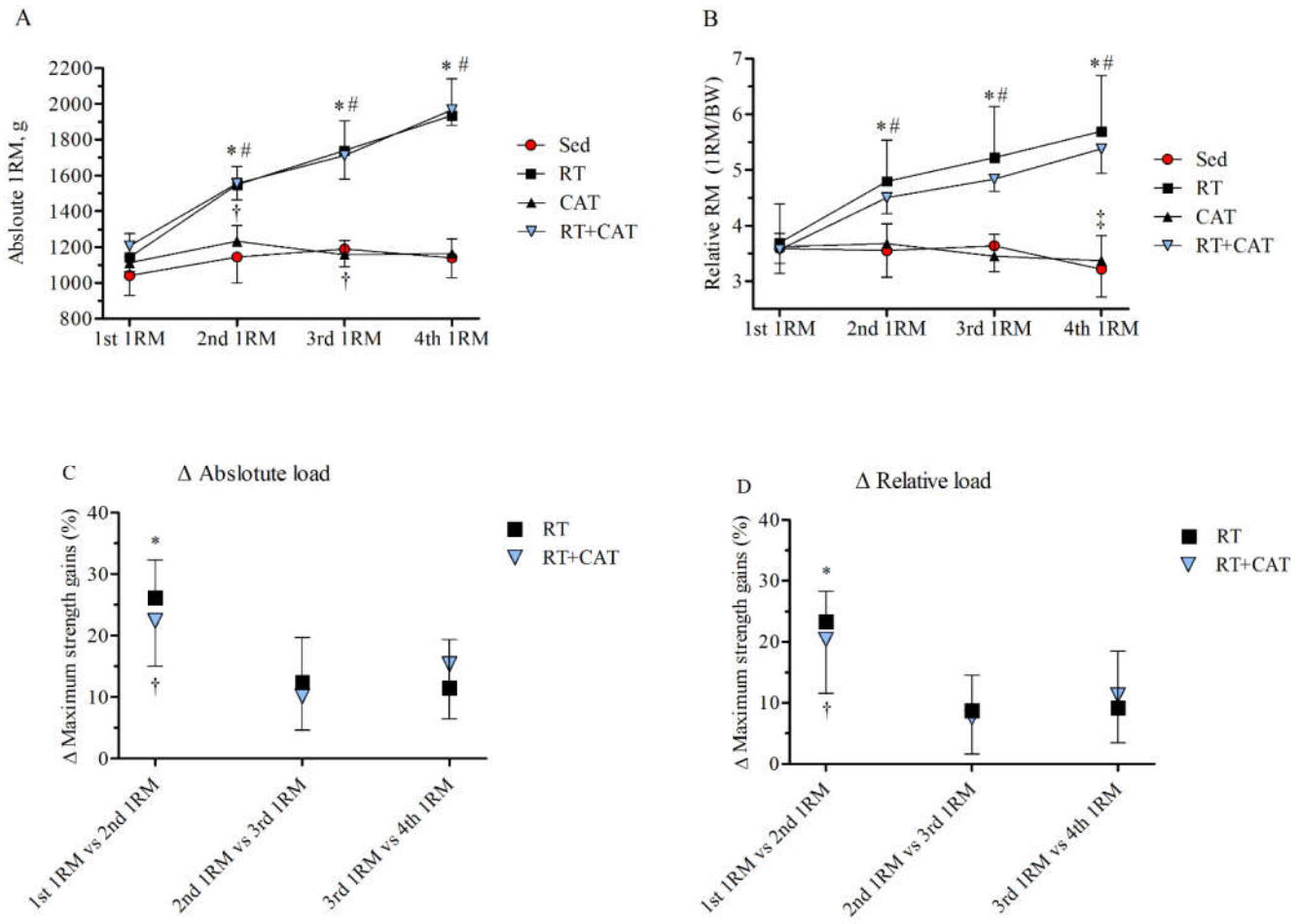
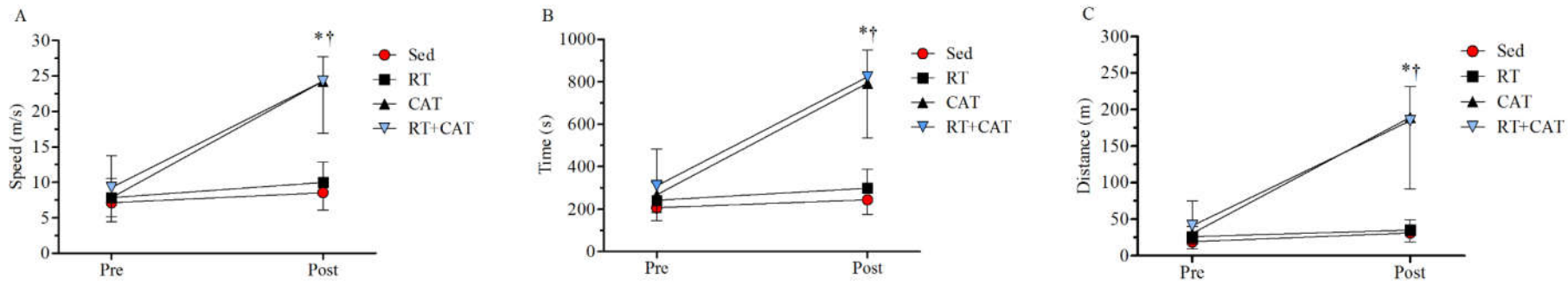

A
Absloute 1RM, g
2200
2000
1800
1600
1400
1200
1000
800
1st 1RM
2nd 1RM
3rd 1RM
4th 1RM
Sed
RT
CAT
RT+CAT
B
Relative RM (1RM/BW)
7
6
5
4
3
C
Δ Absolute load
Δ Maximum strength gains (%)
40
30
20
10
0
1st 1RM vs 2nd 1RM
2nd 1RM vs 3rd 1RM
3rd 1RM vs 4th 1RM
RT
RT+CAT
D
Δ Relative load

Figure 3.


A
Speed (m/s)
30
25
20
15
10
5
0
Pre
Post
*†
Sed
RT
CAT
RT+CAT
B
Time (s)
1000
800
600
400
200
0
Pre
Post
*†
Sed
RT
CAT
RT+CAT
C
Distance (m)
300
250
200
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Pre
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*†
Sed
RT
CAT
RT+CAT

Figure 4.

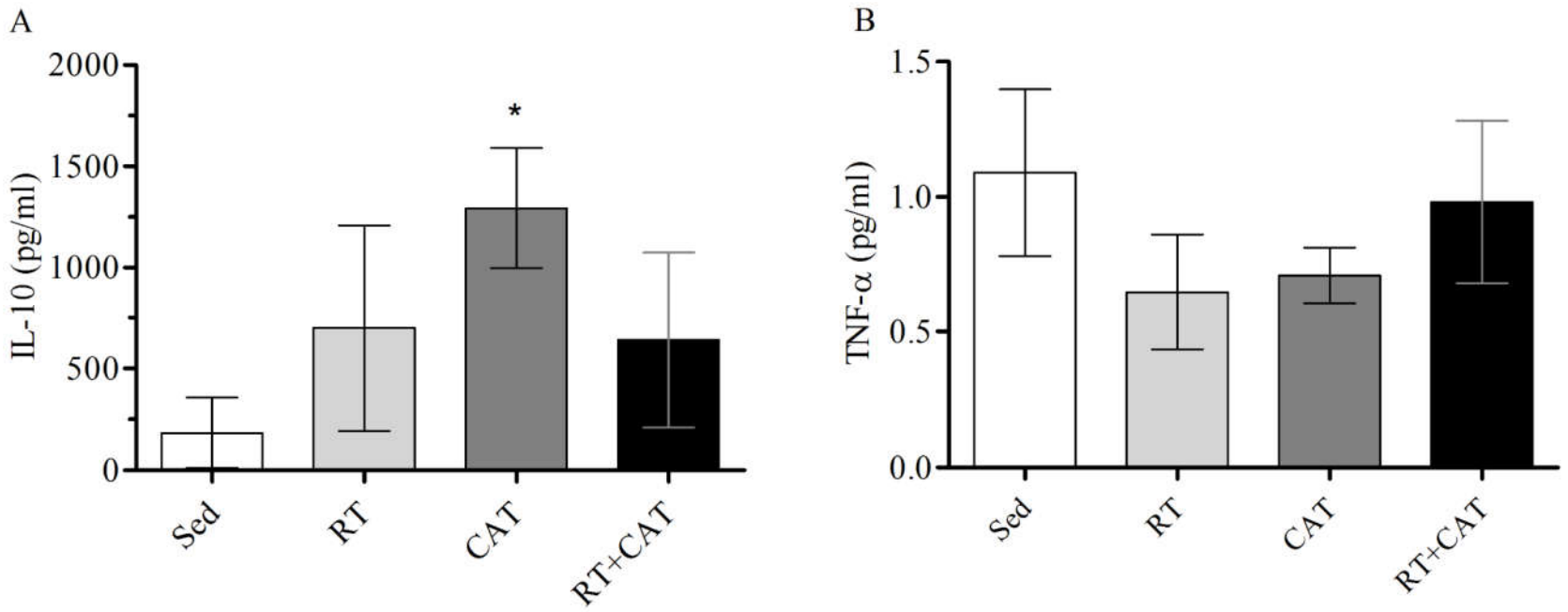

A
2000
1500
1000
500
0
IL-10 (pg/ml)
*
Sed
RT
CAT
RT+CAT
B
1.5
1.0
0.5
0.0
TNF-α (pg/ml)
Sed
RT
CAT
RT+CAT

Figure 5.

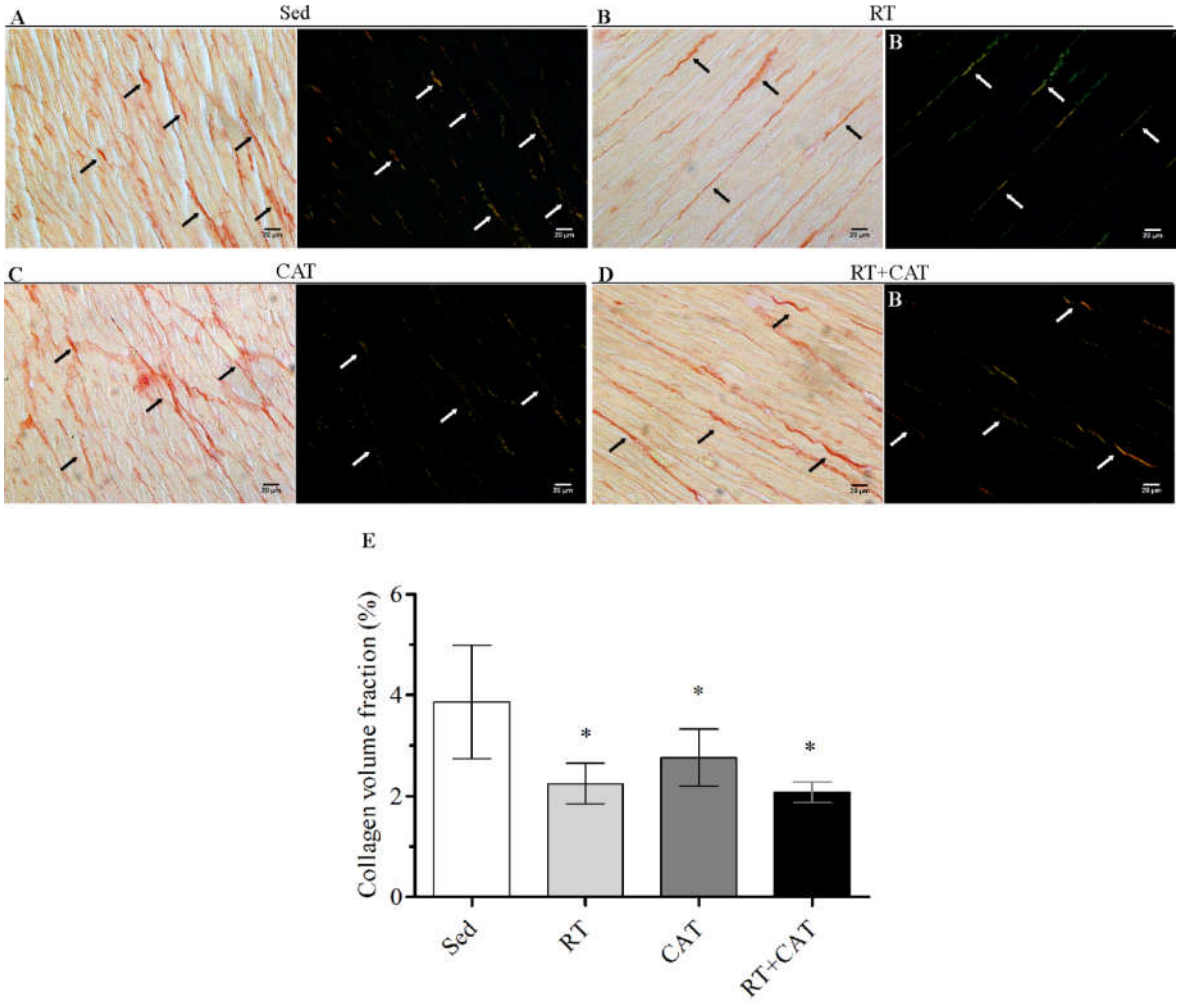
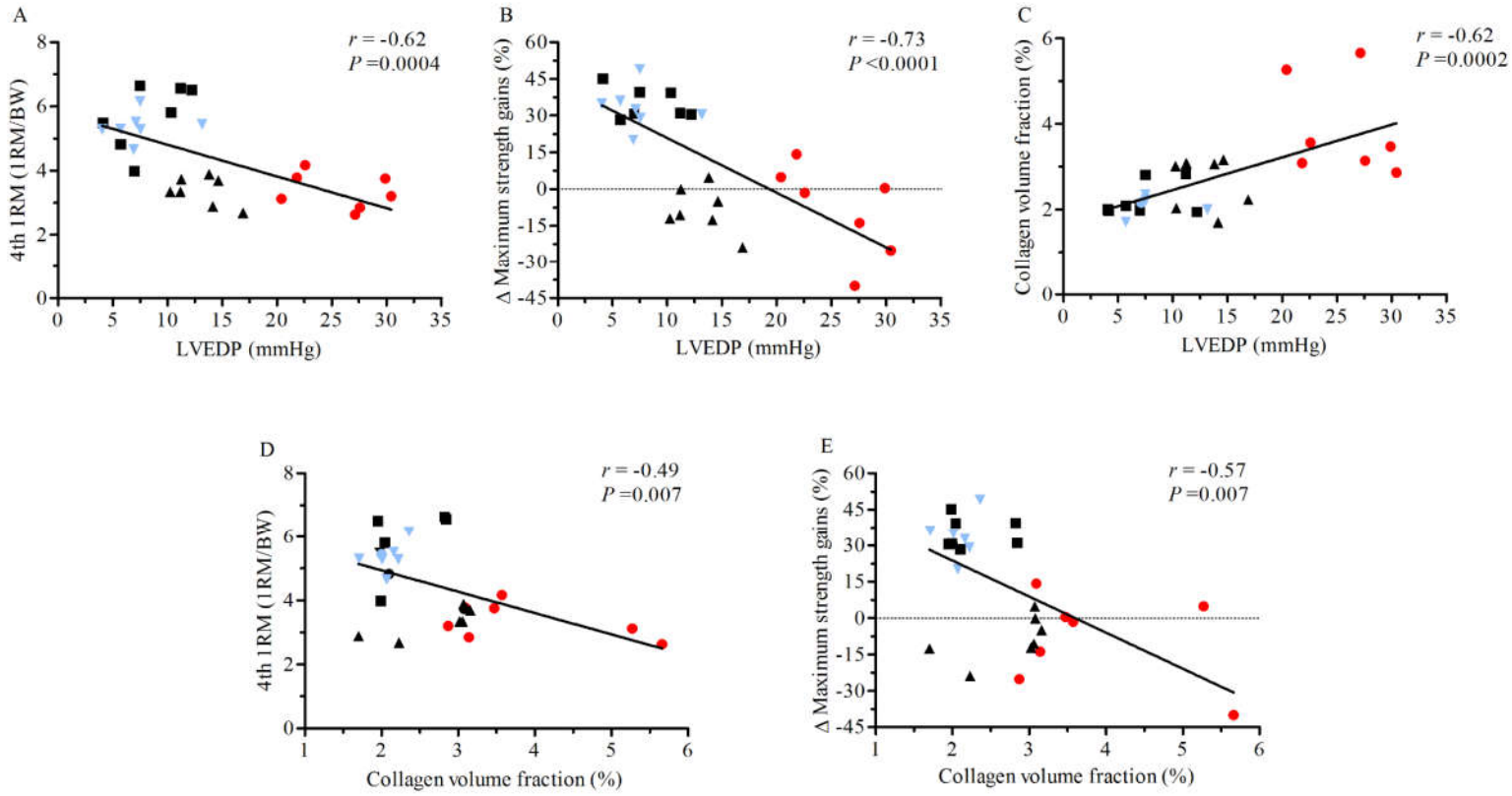

A
Sed
B
RT
B
C
CAT
D
RT+CAT
B
E
Collagen volume fraction (%)
6
4
2
0
*
*
*
Sed
RT
CAT
RT+CAT

Figure 6.



Correlations between the left ventricle end-diastolic pressure (LVEDP) with maximum gain in strength and volume fraction of collagen. red circles (●), Sed; dark square (■), RT; dark triangle (▲), CAT; and blue triangle (▼), RT+CAT. A: LVEDP with 4th 1RM/BW; B: LVEDP with Δ maximum strength gains (%); C: LVEDP with fraction of collagen volume (%); D: fraction of collagen volume with 4th 1RM/BW; E fraction of collagen volume with Δ maximum strength gains (%). Statistical analysis: Pearson's correlation test.

5. Artigo 2

The impact of isolated resistance training or combined with continuous aerobic training on strength gain, hemodynamic function, volume of collagen and functional capacity in healthy and heart failure rats

(Artigo formatado pelas normas da revista *European Journal of Applied Physiology*, fator de impacto: 2,328)

The impact of isolated resistance training or combined with continuous aerobic training on strength gain, hemodynamic function, volume of collagen and functional capacity in healthy and heart failure rats.

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Funding sources

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil.

5.1 Abstract

Purpose: To evaluate the impact of high-intensity resistance training (RT) isolated or combined with continuous aerobic training (CAT) on left ventricular dysfunction, cardiac remodeling, inflammatory markers and volume of series and repetitions (RT) in healthy and in heart failure (HF) rats.

Methods: After five weeks of MI or sham surgery, 41 male Wistar rats (90 days old) were divided into the following groups: sedentary sham (Sed-Sham, n=8), resistance training sham (RT-Sham, n=8), combined exercise sham (RT+CAT-Sham, n=7), sedentary heart failure (Sed-HF, n=6), resistance training heart failure (RT-HF, n=6) and combined exercise heart failure (RT+CAT-HF, n=6). Trained animals were subjected to a RT protocol: 3x/week, 75% to 85% of one repetition maximum (1RM) for 8 weeks. The animals of the combined exercise regimen performed both the RT and CAT.

Results: The Sham and HF trained groups showed the same responsiveness to gain muscle strength and improved functional capacity ($P>0.05$). The left ventricular end-diastolic pressure (LVEDP) and collagen volume fraction (CVF) were attenuated in the infarcted groups subject to RT protocols compared to Sed-HF group ($P<0.05$). The total volume of sets and repetitions showed a close relationship with attenuated LVEDP and the volume of repetitions also influenced the reduction of CVF in HF rats. Regarding the inflammatory markers, no differences were found among groups.

Conclusions: Our results suggest that the balance between intensity, volume of series and repetitions, in the RT, can play an important role in ventricular remodeling and cardiac function in animals with HF and magnitude of responses to muscular strength gains are similar between healthy and infarcted rats.

Keywords: Cardiac function, hemodynamics, muscle strength, collagen, heart failure.

Glossary

+dP/dt_{max} - Maximum positive Left ventricular change in pressure over time

-dP/dt_{max} - Maximum negative Left ventricular change in pressure over time

1RM - Test of one repetition maximum

MI - Myocardial infarction

CAT - Continuous aerobic training

CVF - Collagen volume fraction

DBP - Diastolic blood pressure

ETT - Exercise test tolerance

HF - Heart Failure

HR – Heart Rate

LV - Left ventricle

LVEDP - Left ventricular end-diastolic pressure

LVSP - Left ventricular systolic pressure

LVW/BW - Left ventricular weight-to-body weight

MAP - Mean arterial pressure

MI - Myocardial infarction

MW/BW - Myocardial Weight-to-body weight

PC - Pulmonary congestion

RT - Resistance training

RV - Right ventricle

RVW/BW - Right ventricular weight-to-body weight

SBP - Systolic blood pressure

TNF-α - Tumor necrosis factor – alpha

5.2 Introduction

Resistance training is an important tool in various sports and also is inserted on rehabilitation, but the quantification of dose response is essential to safe and proper prescription independent of the proposal (Wernbom et al. 2007). The optimal dose of exercise for improved state of health is a current topic that deserves attention, since it is indispensable to better comprehend the adjustment of the amount of exercise that should be encouraged or prescribed for obtaining favorable responses and that doubt is greater when prescribing exercise training regimen for patients with cardiovascular disease (Eijsvogel 2015).

Currently, muscle strength is considered an important component of physical ability, has an independent role in preventing chronic diseases whereas muscle weakness is closely related to functional limitations and physical disability. Moreover, the muscle strength presented inverse association for all-cause mortality has been confirmed in different populations such as cardiovascular disease, peripheral artery disease, cancer, kidney disease, chronic obstructive pulmonary disease and others (Volaklis et al. 2015a; Sokka and Hakkinen 2008; Kilgour et al. 2013; Artero et al. 2011; Singh et al. 2010; Puhan et al. 2013; Stenvinkel et al. 2002).

Resistance training is a widely used tool to improve the health condition of healthy individuals or illness. In the last decades, many studies have pointed to the favorable effects of resistance training front of the complex scenario imposed by chronic diseases such as diabetes, hypertension, cancer, stroke and HF (Eves and Plotnikoff 2006; Nascimento Dda et al. 2014; De Backer et al. 2009; Flansbjer et al. 2008; Jewiss et al. 2016). Muscle weakness is appointed as an independent risk factor for mortality in patients with chronic HF (Anker et al. 1997). Moreover, the muscular strength is identified as a long-term predictor of survival in patients with severe HF (Hulsmann et al. 2004).

The suggestions for the practice of resistance training in HF are based on moderate intensity (Selig et al. 2010); few studies have shown the effects of high-intensity resistance training in patients with cardiovascular disease (Toth et al. 2012; Savage et al. 2011) and the training volume has been little explored in resistance training modality for cardiac patients. Resistance training is effective to stimulate muscle hypertrophy and improve strength, but to achieve determined responses is required an adjustment in acute training variables as exercise selection, order, intensity, volume, duration, frequency and rest intervals (American College

of Sports 2009). The choice of volume and intensity in resistance training protocol depends mainly on the proposed objectives for a given population.

Within a translational vision, there are few studies that used squat exercise model for rats, among these the vast majority employed to moderate intensity in the experimental design (Barauna et al. 2007; Krisan et al. 2004; Mostarda et al. 2014). The adaptations of the heart muscle in the experimental model of squats have been reported and these results show that the model can be employed to investigate the effects of interventions in the context of cardiovascular diseases (Barauna et al. 2005). Accordingly, we hypothesized that high-intensity resistance training alone or combined with aerobic training may produce similar responses between healthy and infarcted animals in relation to the gain strength, volume and repetitions and maximum tolerance test to physical exercise (ETT). Therefore, this study aims to advance knowledge regarding the high-intensity resistance training alone or combined with continuous aerobic training on hemodynamic parameters, inflammatory markers, collagen deposition and functional capacity in animals post MI and healthy submitted to the adapted model of squat apparatus for small animals.

5.3 METHODS

This study followed the recommendations described in ARRIVE (Animals in Research: Reporting In Vivo Experiments) guidelines (Kilkenny et al. 2010).

Sample, Experimental Design and Ethical Approval

The experiments in this study followed the rules established by the guide for care and use of animals in experiments published by National Institute of Health (NIH publication no. 41-87, revised in 2011). All the procedures defined in this study were approved by the Ethics Committee in Research of Universidade Federal de Ciências da Saúde de Porto Alegre (protocol 199-13).

Sixty- five male Wistar rats (220 to 270g, 90 days of age) were obtained from the breeding animal unit of the Universidade Federal de Ciências da Saúde de Porto Alegre and were housed under standard conditions (food and water ad libitum, a light and dark cycle of 12:12 hours and 22 ° C ambient temperature). Following myocardial infarction (MI) or sham surgery, the animals were kept for a period of five weeks post-surgically (time needed to reach the development of a HF state) (Francis et al. 2001; Pfeffer et al. 1979). The animals were allocated into 6 groups: Sed-Sham, RT-Sham, RT+CAT-Sham, Sed-HF, RT-HF and RT+ CAT-HF. Figure 1 depicts the timeline in relation to the experimental procedures.

Induction of acute myocardial infarction or sham surgery

The animals were weighed and anesthetized by inhalation with 2% isofluorane (Isofluorine, 100 ml - Cristália) in 100% oxygen (Munkvik et al. 2011) in a clear glass bell jar and were immediately artificially ventilated afterwards (Samway VR 15, 60 breaths / minute and a fraction of 100% oxygen inspired). Following thoracotomy, the ligation of the coronary artery was performed to induce MI as described previously (Capalonga et al. 2016; Jaenisch et al. 2011; Nunes et al. 2015). To reduce the algic and inflammatory effects subsequent to surgery, analgesic Turbogesic® (butorphanol) 0.5 mg / kg was administered intraperitoneally 12/12h during the first 24h postoperatively. The sham animals underwent the same procedure without artery ligation, as described previously (de Leon et al. 2011).

Resistance Training

Adaptation

Five weeks after performing surgery, the animals of the MI training groups were subjected to a familiarization period with the adapted squat apparatus (Krisan et al. 2004). The animals were placed in the apparatus and encouraged to perform 1 bout of 5 to 10 repetitions with 40% to 60% of body weight for 3 days for the first week.

1RM test

To determine training load, all animals were subjected to the 1RM test. The 1RM test has been widely used in experimental designs of resistance training as previously described (Barauna et al. 2007; Krisan et al. 2004; Stefani et al. 2014).

RT protocol

The methodology adopted for the execution of RT protocol utilized an electric stimulus (4-5 mA, 1 s duration, with a 3 s interval between each repetition) applied to the rats' tails through an adapted surface electrode (Alves et al. 2014). The training protocol lasted for 8 weeks with 4 bouts of 6 to 10 repetitions with 90 s rest periods between each series three times per week with 75% load and 85% of the 1RM method adapted from Barauna et al. (Barauna et al. 2007). Therefore, the animals were exercised with 75% of 1RM in the first week and 85% of 1RM during the second week of training. The intensities of this protocol were classified as vigorous at near-maximum to maximum (high-intensity) according to the guidelines of the American College of Sports Medicine (ACSM) (Garber et al. 2011). The training program follows the recommendations of the American Physiological Society (APS) (Kregel 2006).

Total volume of sets and repetitions of resistance training

The total volume of the sets and repetitions was calculated for each animal. Only were added volumes of the animals completed the exercise protocol. After the sum of the series and repeats, volumes were statistically analyzed by comparison of the mean for each group.

Continuous Aerobic Training

Adaptation treadmill / exercise test tolerance / incremental load test

Five weeks after surgery, MI or sham surgery animals of the training groups were subjected to a period of adaptation to the treadmill. The rats were placed on the treadmill (0%

inclination) and encouraged to perform a running exercise at a speed of 10m / min for five minutes on the first and second day and ten minutes on the third, fourth and fifth days. The animals were subjected to an exercise tolerance test (ETT) five weeks after MI surgery and after eight weeks of training. The animals were adapted to an ergometer for 5 days (10 minutes / day) before the tests. The protocol employed consisted of a run on the treadmill with an inclination of 15 ° until exhaustion. The protocol began with a speed of 5 m / minute and there was an increment of 5 m / minute every 3 minutes until exhaustion of the animal (Ferreira et al. 2007).

Continuous aerobic training protocol

The training protocol lasted for eight weeks, with 50 minute runs 3 times per week at one 10m / minute speed, which was progressively increased up to 15m / minute (0% inclination) over the proceeding weeks until the end of training. The training load was applied to 60% of the maximum speed obtained in the maximum tolerance treadmill test. The training program followed the recommendations of the American Physiological Society (APS) (Kregel 2006).

Protocol of combined exercise training

The Animals of the RT group combined with CAT initially performed the RT and immediately at the end of training, the animals were subjected to CAT.

Evaluation of Hemodynamic Function

Surgical preparation and hemodynamic evaluation

The recordings were performed 48 hours after the end of the training protocol. The animals were anesthetized with Ketamine (80 mg/kg, i.p.) + Xylazine (12 mg / kg, i.p.) and intubated (Gelko - 14G). A polyethylene catheter model P50 was introduced into the left ventricle (LV) via the right carotid artery. Blood pressure was recorded for 5 minutes immediately following the catheter being positioned within the LV to perform the registration of the ventricular pressure. Data were recorded through a pressure transducer (strain-gauge, Narco Biosystem Miniature Pulse Transducer RP-155, Houston, Texas, USA) coupled to a pressure amplifier (Stoelting, wood dated, Illinois, USA). The analog pressure signals were digitized by the data acquisition system (CODAS-Data Acquisition System, Akron, OH,

USA) with a sampling rate of 2.000 Hz. This data was then used to determine the diastolic blood pressure (DBP), systolic blood pressure (SBP), mean arterial pressure (MAP), heart rate (HR), left ventricular systolic pressure (LVSP), end-diastolic pressure of the left ventricle (LVEDP) and maximum positive left ventricular change in pressure over time ($+dP/dt_{max}$) as well as maximum negative left ventricular change in pressure over time ($-dP/dt_{max}$) (Alves et al. 2014).

Blood sample and collection of tissue

Blood collection was conducted with the tube already positioned in the left carotid artery and samples were taken in two 1.5 ml tubes containing 3.2% sodium citrate (1: 9 v / v) and next centrifuged at 500 x g at 4 ° C. The plasma was stored at -22 ° C. Immediately after the hemodynamic assessment and blood collection, the animals were sacrificed by decapitation and the heart, lung and liver tissues were harvested (Alves et al. 2014).

Cardiac hypertrophy, pulmonary and hepatic congestion

The rat hearts were removed and weighed without blood in the chamber and without the atriums. The LV and right ventricle (RV) were dissected from each heart, separated and weighed. For the determination of cardiac hypertrophy, the organs were expressed as a ratio to body weight (tissue weight / body weight - mg/g) (Alves et al. 2014; Fernandes et al. 2011; Nunes et al. 2015). To determine lung and liver congestion, the liver and lungs of each animal were removed, weighed and subsequently dried (80 ° C) for 48 hours and weighed again to determine the volume of water. The proportions of wet and dry weight of tissue were used to establish the percentage of water in the tissues as an indication of pulmonary congestion (PC) and hepatic congestion. To evaluate congestion, the following formula was utilized:

$$Congestion\ (\%H_2O) = \frac{(Wet\ weig\ \ t \quad Dry\ weig\ \ t)}{(Wet\ weig\ \ t\ x\ 100)}$$

Determining the size of the infarcted area

Directly following the calculation of the weight of the heart, the left ventricles were filled with a latex pad and submerged in buffered formaldehyde for 10% over 24 h for subsequent analysis of the size of the infarcted area. The size of the infarcted area was determined by histological evaluation adapted from the method previously described by Martinez et al. (Martinez et al. 2011). The measurements were performed in the mid-ventricular slices (5-6 mm from the tip) under the assumption that cuts from that point

exhibited a close linear relationship with the sum of the measures of all heart cuts (Oh et al. 1993). Therefore, 6 mm thick of medium-ventricular cut sections were stained with Masson's trichrome. The ImageJ software version 1:49 was used to obtain the length of the whole circumference of the endocardium and the endocardium tracking the length of the infarcted region formed from two medium-ventricular sections of the LV via manual planimetry. The fraction of the infarcted area of the LV was calculated from these measurements. All measurements of the infarct areas were by a single technician who was blinded in relation to the hemodynamic data and work of the group.

Analysis of interstitial collagen deposition in the heart muscle

Cross cuts were performed on the LV and these fragments were fixed in 10% buffered formaldehyde for subsequent inclusion in paraffin, a method adapted previously (Matsubara et al. 2000; Whittaker et al. 1989).

Histological sections of 6 microns were stained with picrosirius Red (PSR) and were employed for measurements of CVF present in the interstices of LV cardiac tissue. The readings were performed using polarized light (Whittaker et al. 1989) with an Olympus microscope attached to a video camera that sent a digital image to a computer with the image analysis program, Image Pro-plus (Media Cyberetics, Silver Spring, Maryland, USA). It was analyzed at 40 fields per ventricle with a 40X objective. The fields selected were located far from the infarcted area and distant from the pericardial region as previously described (Alves et al. 2014). The CVF was defined as the sum of all areas of interstitial collagen tissue (highlighted by polarization) divided by total tissue area (Matsubara et al. 2000).

Determination of plasmatic levels of interleukins

After hemodynamic recording, blood collection was performed to determine the plasma concentrations of TNF-α and IL-10. The markers were assessed through a Milliplex Map kit (RCYTO-80K, Millipore - Billerica, MA, USA) for cytokine rats. Milliplex Map is based on the Luminex xMAP technology following the manufacturer's recommendations. These techniques have already been conducted in other experiments in our laboratory (Alves et al. 2014; Nunes et al. 2008). All cytokines are presented in pg/ml.

Statistical analysis

Each variable and group were expressed as mean ± SD. The Shapiro-Wilk test was carried out to evaluate the normality of all variables. We used two-way analysis of variance (ANOVA) followed by a Tukey's post hoc test to compare variables between groups (body weight, infarcted area size, cardiac hypertrophy, pulmonary and hepatic congestion, hemodynamic variables, maximum percentage gain strength, interleukin-10 and TNF-α and fraction of collagen content). A Two-way repeated measures ANOVA with subsequent Tukey's post hoc test was employed to compare the strength gain between groups at different experimental times and also to compare speed, distance and time in the ETT. Pearson's correlation test was applied to examine the relationship amongst the volume of sets and repetitions with the hemodynamic parameters and deposition of interstitial collagen. Values of $P < 0.05$ were considered significant. Statistical and construction of graphical analysis was with GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA) for Windows. Sigmaplot version 12.0 (Systat Software Inc., San Jose, CA, USA) software was utilized for complementary statistical analysis.

5.4 RESULTS

Mortality, sample and adverse effects

The mortality of the animals submitted to MI was ~42% (15/36) and the sham surgery was ~ 7% (2/29). Statistical analysis was performed only with animals that completed the experimental protocol. The study only consisted of animals with a large infarct area (≥ 40%). Some animals were excluded due to lack of hemodynamic record: Sed-HF (1), Sed-Sham (1), RT-HF (1) and other animals did not reach at least 75% of the total exercise protocol physical: RT-Sham (1), RT+CAT-HF (1), RT+CAT-Sham (2). Following exclusion of animals, all experimental groups were divided as follows: Sed-Sham, n = 8, RT-Sham, n = 8, RT+CAT-Sham, n = 7, Sed-HF, n = 6, RT-HF, n = 6 and RT+CAT-HF, n = 6.

Morphological variables, pulmonary and hepatic congestion

All data are summarized in Table 1. The initial and final body weight of the animals was not different between groups, as well as areas of infarction. These data suggest that all groups started the protocols in a similar state. Left ventricular hypertrophy (LVW/BW) was lower in Sed-Sham and RT-Sham groups when compared to groups with HF, the RT+CAT-Sham group showed lower hypertrophy compared to the RT+CAT-HF. No differences were

observed between the groups with HF. The hypertrophy of the right ventricle (RVW/BW) was greater in the Sed-HF group compared to all groups, no differences were observed between the trained groups with HF when compared to Sham groups. These results demonstrate that high-intensity RT protocol alone or combined with CAT was able to attenuate the right ventricle hypertrophy in animals with HF. Additionally, the ratio RVW/BW was lower in RT+CAT-HF group compared to RT-HF group, possibly this result is associated with the sum effect of combined exercise protocols. The hypertrophy of the total myocardium (MW/BW) was greater in the Sed-HF group compared with all the Sham groups and the RT+CAT-HF group. The RT-HF group also showed difference in this variable, when compared to the sham groups. The RT+CAT-HF group showed a difference in the ratio of MW/BW when compared to RT-Sham group, but no difference was observed when compared with the Sed-Sham and RT+CAT-Sham groups. Regarding pulmonary congestion (PC, %Water), it was observed an increase in the Sed-HF group compared to all groups. These results suggest that exercise protocols were able to attenuate pulmonary congestion animals with HF. Additionally the RT-HF group showed no difference in the PC (% H_2O) when compared to the Sham groups and the RT+CAT-HF group showed no difference for this variable when compared to Sed-Sham and RT+CAT-Sham groups.

Hemodynamic evaluation

All data are summarized in Table 2. No changes were observed in the following hemodynamic variables: HR, SBP, DBP and MAP between groups. However, an increase in the LVEDP in the Sed-HF group was observed when compared to all groups. The RT-HF group had higher LVEDP compared to Sham groups, but the RT+CAT-HF group showed no differences when compared to Sham groups, these results demonstrate that the combined exercise can be effective to positively modulate an important marker of cardiac dysfunction. The LVSP was lower in Sed-HF group compared to the RT+CAT-HF group. No differences were observed between Sham groups and trained HF groups. The $+dP/dt_{max}$ was lower in Sed-HF group compared to the Sham groups and the RT+CAT-HF group. The groups trained with HF showed a reduction in $+dP/dt_{max}$ when compared to Sham trained groups, but no differences were observed when compared with the Sed-sham group. Regarding the $-dP/dt_{max}$ no differences were observed between the HF groups, but as expected the Sed-HF group showed lower $-dP/dt_{max}$ compared to Sham groups. Additionally, the groups trained with HF

showed lower values when compared to Sham trained groups, but no differences when compared with the Sed-Sham group.

Number of series and repetitions

Figure 2 A-B demonstrates the exposure of the animals with infarction or sham trained in relation to the sum total of the series and repetitions performed for 8 weeks in the isolated or combined resistance training protocol. In the graph 2A is observed that the sham groups has a greatest amount of volume of series compared to groups with HF. However, in the graph 2B, the RT-Sham and RT+CAT-HF groups exhibited a greater number of repetitions sum when compared to RT-HF group. These findings suggest that the group of infarcted animals submitted to combined exercise protocols did not lose their capacity to adapt to exercise because of MI.

Maximum strength gain

Figure 3 shows the absolute (A) and relative (B) strength gains related to groups submitted to training protocols in the segment over time (1st 1RM, 2nd 1RM, 3rd 1RM and 4th 1RM). No differences were observed in the 1st 1RM test between the experimental groups for both absolute as relative loads. This result shows that the animals were in similar conditions at the beginning of the training protocol. The groups submitted to resistance training protocols alone or combined with aerobic training presented gain progressive strength compared to the initial test (1st 1RM) in both absolute and relative loads. Regarding the strength gain between the groups, it was observed that the groups exposed to the training protocol showed increased strength in the 2nd, 3rd and 4th 1RM test when compared to sedentary groups in both absolute and relative loads. These results suggest that animals with HF have not lost the capacity to gain strength and showed similar values compared to trained animals healthy.

Magnitude strength gain between the percentage differences of maximum load tests

Figure 3 shows the percentage differences between the 1RM tests absolute (C) and relative (D) concerning the loads of the groups submitted to resistance training protocols. In Figure 2C and 2D, we can observe that the greatest percentage difference between the tests occurs within the first two weeks of training (1st vs 2nd 1RM test) when compared with the

percentage differences of 2nd vs 3rd 1RM test and 3rd vs 4th 1RM test, both in absolute and in relative loads. No changes are observed between the percentage differences of 2nd vs 3rd 1RM test compared to the 3rd vs 4th 1RM test. These results demonstrate that the greatest magnitude in strength gain in healthy and HF animals, in the adapted model squat for small animals, occurs during the first two weeks, in the weeks following the strength gains occur, but on a smaller scale. Furthermore, the magnitude of the strength gain of animals with MI compared to healthy was not changed due to the scenario of cardiac dysfunction.

Exercise tolerance teste (ETT)

Figure 4A (speed), 4B (Time) and 4C (Distance) shows the variables analyzed during the ETT of pre- and post- intervention of the experimental groups. Interestingly, at the moment pre ETT, it was observed that the Sham groups showed greater speed reached when compared to Sed-HF and RT- HF groups. In the post intervention test, it was observed that RT+CAT-Sham group had higher speed in the test when compared to all groups. The RT+CAT-HF group presents greater speed in the pos ETT when compared to the sedentary groups and RT-HF group, no differences for the RT-Sham group. We also observed that the RT-Sham and Sed-Sham groups reached higher speed in the post ETT when compared with the Sed-HF and RT-HF groups. Additionally, in the intra-group comparison pre and post ETT were observed differences in speed reached within the following groups: RT-Sham, RT+CAT-Sham and RT+CAT-HF. In the chart 4B is displayed the total time in seconds of pre and post ETT. The RT+CAT-Sham group exhibited greater speed at pre ETT when compared to groups with HF, but no difference in values when compared to the Sed and RT Sham groups. The Sed-Sham and RT-Sham groups also exhibit greater in total time of pre ETT when compared with the Sed-HF and RT-HF groups. Regarding the post ETT, it was possible observe that the groups trained with combined exercise (RT+CAT) showed higher values in total time when compared to all other groups. However, the RT-Sham group reaches greater time in the post ETT when compared to sedentary and RT-HF groups, The Sed-Sham group recorded greater time in the post ETT when compared with the Sed-HF and RT-HF groups. In the comparison intra-groups, it was observed that the RT-Sham, RT+CAT-Sham and RT+CAT-HF groups showed higher values in time between the pre and post ETT. Regarding the covered distance (graph 4C), it was observed that the RT+CAT-Sham group showed greater distance in the pre ETT when compared with the Sed-HF and RT-HF groups. In the post ETT was observed that the combined exercise groups showed greater distance

covered when compared with the Sed-HF, RT-HF, Sed-Sham and RT-Sham groups. Additionally, RT-Sham group achieved a greater distance in the post test when compared with the Sed-HF, RT-HF and Sed-Sham groups. Furthermore, sedentary groups showed differences in distance values in the post test when compared between themselves. The RT-Sham, RT+CAT-Sham and RT+CAT-HF groups presented higher values in the distance traveled between the pre and post ETT. These results suggest that the differences found among the tests in the Sham groups without adaptation to CAT, may be related to the absence of limiting factors of HF.

Interstitial collagen volume fraction

Figure 5 shows the statistical analysis of collagen volume fraction of the experimental groups trained and untrained. The results show that the sedentary group submitted to MI showed fibrosis accumulation when compared to all groups. Moreover, the collagen volume fraction of the trained groups with HF was significantly higher than the sham groups. Interestingly, these data revealed that RT alone or combined with CAT was able to help in attenuating myocardial fibrosis in the animals submitted to MI.

Inflammatory markers

As demonstrated in Figure 6 we found no statistical difference in inflammatory markers among groups for both IL-10 and TNF-α. These results demonstrate that high-intensity resistance training was not able to modulate the inflammatory markers in this study in all experimental groups.

Correlations

Figure 7A, 7B and 7C illustrates the correlation between LVEDP with volumes of sets and repetitions of RT in the animals subjected to MI. It is also demonstrated the correlation between volume of collagen fraction to the volume of repetitions of the RT. We observed a significant negative correlation between increased exposure in sets and repetitions RT protocol with LVEDP in animals with HF. Additionally, it was observed that the greatest exposition in the number of repetitions of the RT protocol also provided an attenuation of collagen volume fraction. These results demonstrate that increased exposure of the series and repetitions of a high-intensity RT protocol can positively modulate a marker of cardiac dysfunction and minimize negative adaptations of cardiac remodeling.

5.5 Discussion

This study was designed to test the effects of exposure to the number of sets and repetitions of high-intensity resistance training protocol in healthy animals and submitted to MI. The main results of this study demonstrate that both the isolated RT protocol or combined with the CAT were effective in improving parameters of ventricular dysfunction and cardiac remodeling. Another outcome found was that high-intensity employed in resistance training protocol produced similar strength gains between healthy and infarcted rats, demonstrating that animals with MI do not lose their capacity to gain strength and may have additional benefits from these effects. We also demonstrated that the volume of sets and repetitions of the training protocol had influence on the LVEDP (marker of cardiac dysfunction) both in the group RT alone and in the combined group. In addition, the volume of repetitions also had a positive influence on cardiac remodeling of animals with HF subjected to high-intensity resistance training protocols.

Resistance training model for small animals have been used in the last years and demonstrated positive effects on muscle (Tamaki et al. 1992) and cardiovascular (Barauna et al. 2005) adaptations in healthy animals, but currently this model presented interesting responses in animals with acute myocardial infarction (Alves et al. 2014) and diabetics (Mostarda et al. 2014). The Attenuation of hypertrophy of the right ventricle (RVW/ BW) has been observed in respiratory muscle training (Jaenisch et al. 2011) and in moderate-intensity resistance training (Alves et al. 2014) in animals with HF. These findings were also found in this study, it was observed that animals post MI and subject to the RT protocol exhibited diminution in the ratio of RVW/BW, but with more pronounced effects for groups with combined exercise (RT+CAT-HF). The pulmonary congestion is considered a major problem in the course of HF, renal failure and pulmonary hypertension (Yang et al. 2010). Regarding to our study, the results show that the infarcted animals submitted to training protocols exhibited attenuation of pulmonary congestion and this outcome was observed in other studies with different protocols (Nunes et al. 2015; Jaenisch et al. 2011).

The impact of physical exercise on systolic and diastolic function after myocardial infarction has been investigated both in the clinical (Fontes-Carvalho et al. 2015) as well as in experimental model of HF (Alves et al. 2014; Nunes et al. 2015; Nunes et al. 2008). In the experimental model of coronary artery occlusion, it is common to observe the presence of cardiac dysfunction in these animals (Musch et al. 2002). Interestingly, the exercise protocols of this study were able to attenuate the LVEDP of animals with HF. This result demonstrates

the effectiveness of the training in positively modulate an important marker of cardiac dysfunction. Furthermore, the volume of sets and repetitions of resistance training showed a significant close relation with the reduction of LVEDP, other interventions also have proved to be effective to improve this important predictor of HF (Jaenisch et al. 2011; Nunes et al. 2015; Nunes et al. 2008; Almeida et al. 2014).

Abnormalities in systolic function in patients with heart failure can occur due to reduced stroke volume and cardiac output resulting from a reduced LV contractility (Shammas et al. 2007). In the present study we observed that animals submitted to the high-intensity resistance training protocol combined with aerobic training showed improvement in LVSP, this result is also accompanied by a positive response in relation to an increase of $+dP/dt_{max}$ for the same group. However, in the clinical setting, in the intention-to-treat analysis, no significant improvements were observed in systolic function in patients with HF who performed aerobic training or combined aerobic with resistance training (Mandic et al. 2009), another study also showed that the association of aerobic exercise with resistance training was not able to significantly improve systolic and diastolic function, even with the presence of improvement of parameters related to the capacity to exercise (Fontes-Carvalho et al. 2015). On the other hand, studies in animal models of MI and HF reported improvement in left ventricular function through the respiratory muscle training, continuous aerobic training and interval training (Almeida et al. 2014; Jaenisch et al. 2011; Nunes et al. 2015; de Moraes et al. 2015). Additionally, Mostarda et al demonstrated that low-intensity resistance training induces improvement in systolic function in rats with diabetes (Mostarda et al. 2014).

The quantification of dose-response between training variables and the obtained responses are essentials for an appropriate prescription of resistance training (Wernbom et al. 2007). In our study, we evaluated the number of sets and repetitions performed throughout the experiment and animals trained of the sham groups had a higher sum in the number of sets than the trained groups with HF, already the number of repetitions were higher in RT-Sham group and exercise combined with HF. Therefore, responses to the training volume did not influence the ability of animals, especially the infarcted groups, to obtain strength gains during the protocol, these findings are consistent with a study of Savage and collaborators that elegantly demonstrated the increase of similar strength among patients with HF and sedentary individuals control submitted to controls RT protocol (Savage et al. 2011).

In recent years, the loss of muscle mass and muscle strength has been widely discussed in a variety of populations (Volaklis et al. 2015a; Neves et al. 2016). Additionally,

muscle weakness in patients with HF serves as a predictor of mortality, while the gain in survival is associated with increased muscle strength (Anker et al. 1997; Hulsmann et al. 2004). Our study showed that animals with HF submitted to high-intensity resistance training protocols progressively increased strength as well as healthy animals, these findings are strengthened when we observe gain the percentage of strength gain among the 1RM test. Moreover, the magnitudes of initial strength gain and subsequent strength gains are similar between the groups trained with HF and animals sedentary controls, we can also emphasize that the greatest percentage of strength gain is concentrated in the first two weeks, subsequently gains occur on a smaller scale with no differences between them, these results may justify the absence of a 1RM test at the end of the protocol. In a study conducted in our laboratory, showed that animals trained with moderate intensity RT also increases the strength indices with no difference between healthy and infarcted groups (Alves et al. 2014). Another study showed that the loss of strength capability is directly associated with mortality in severe cancer cachexia model in rats and the short-term moderate intensity resistance training promoted only modest effects during the progression of cachexia (Neves et al. 2016).

The functional capacity of animals subjected to RT protocols was increased in groups with combined exercise and in the RT-Sham group, we emphasize that the healthy and infarcted groups, subject to the combined protocol exercise, showed no differences in speed, time and distance test, showing again that animals after MI do not lose the ability to improve the ETT. The improved functional capacity was also observed in other study with different exercise protocols (continuous aerobic training and interval aerobic training), but in the same MI model (Nunes et al. 2015; Masson et al. 2016). Interestingly, RT- sham group showed an increase in speed values, time and distance, and these results are similar to Jankowska et al findings showed that 12 weeks of progressive resistance training for quadriceps improved the exercise capacity of patients with heart failure, were reported increases in distance covered (6MWT) and time in the cardiopulmonary exercise testing (Jankowska et al. 2008).

Cardiac stress can induce morphological, structural and functional changes in the heart and myocardial infarction may result in progressive remodeling and advance to the backdrop of the HF (Piek et al. 2016). After MI are triggered repair actions that promote excessive increase of collagen which leads to damages in cardiac distensibility, diastolic and systolic function and electrical transmission that leads to arrhythmia and therefore the case of HF (van Putten et al. 2016). The reverse cardiac remodeling in HF through physical exercise is a current topic and aerobic exercise has been shown to be significant to induce reverse cardiac

remodeling, but resistance training alone or combined with aerobic training seems to have no effect on the reverse remodeling (Adams and Niebauer 2015). In our study, we observed that high-intensity resistance training was able to attenuate the increase in interstitial collagen in the hearts of animals with HF, these results are supported by other studies, in the animal model, that also showed improvement in cardiac remodeling through the moderate-intensity RT (Alves et al. 2014), aerobic exercise training (Guizoni et al. 2016; Nunes et al. 2015; Xu et al. 2008b) and aerobic exercise training combined with losartan (Xu et al. 2008a). Furthermore, we observed that the total number of repetitions performed in the resistance training protocol is closely related to interstitial collagen deposition attenuation of animals subjected to RT alone or combined with continuous aerobic training.

In human studies, the aerobic exercise training has demonstrated beneficial effects on cardiac remodeling in patients post MI that starting training around of 1 week or after this period (Haykowsky et al. 2011). Another study showed that patients after myocardial infarction submitted to aerobic training twice a week for three months have positive cardiac remodeling assessed by magnetic resonance imaging (Izeli et al. 2016). Few studies have evaluated the effects of resistance training on cardiac remodeling, these few findings reported that the combined training (aerobic plus resistance) or RT alone do not improve or damage the cardiovascular remodeling (Haykowsky et al. 2007; Chen et al. 2012).

Cytokines have been linked to the development of HF, having multiple biological effects that can lead to left ventricular dysfunction, pulmonary edema, and adverse left ventricular remodeling (On et al. 2014). In addition, inflammatory cytokines have shown an important role in the context of muscle cachexia, which is characterized by the development of muscle weakness, weight loss, and muscle atrophy in a variety of serious illnesses (Zhou et al. 2016). We did not find improvement of inflammatory markers of animals subjected to the training protocols. Possibly the high-intensity resistance training has no positive effects on these parameters in experimental model of MI. On the other hand, the low to moderate intensity RT has proven to be a safe and effective tool in non-exacerbate inflammation in patients with coronary artery disease (CAD) (Volaklis et al. 2015b). In the experimental model of the HF aerobic exercise training demonstrated potent anti-inflammatory action in animals subjected to eight weeks of swimming (Nunes et al. 2008) The anti-inflammatory effects of exercise in patients with HF has been widely reported in the literature, but these studies are mostly based on the response of aerobic exercise (Niebauer 2008; de Meirelles et al. 2014; Gielen et al. 2003).

The limitation of this study was the lack of an analysis of tissue inflammatory markers that could explain the inflammation condition of lung, skeletal muscle and cardiac muscle. Another important point would be to evaluate the family of microRNAs that are associated to cardiac fibrosis and are potent regulators of gene expression in pathological cardiac hypertrophy. We believe that the use of a fatigue marker could help to understand, more precisely, the limits reached during physical exercise protocols, such as dosage of HSP-70

In summary, the present study demonstrated that high-intensity resistance training protocol had a positive influence on the ability to gain strength and improve the functional capacity of animals with HF. Moreover, did not occur differences in gain strength and functional capacity among animals with HF and healthy, demonstrating that the high-intensity RT protocols isolated and combined with CAT are not harmful in the experimental model of HF induced by MI, especially in animal with large infarct area. Additionally, the total volume of sets and repetitions of isolated or combined resistance training with aerobic exercise training influenced the improved cardiac function and cardiac remodeling in animals with HF, but did not alter the inflammatory parameters analyzed. These data demonstrate that the dose of high-intensity employed in the resistance training protocol, in the experimental model of HF, can directly interferes in the volume of sets and repetitions. These findings may have positively influenced some responses in relation to the cardiac function and cardiac remodeling, but is still needed a better fit of the variables of physical training to achieve positive responses in the field of inflammatory markers.

Acknowledgements

We are grateful to Prof. Luís Fernando Deresz, Terezinha Stein, Rosalva Theresa Meurer, Ignês Cristiane de Souza Paiva and Mário Pereira (in memoriam) for support during this study.

Authors' contributions

Design and development: JPA, RBN, DCF, RBJ, GPS e PD. Development of the experiments: JPA, RBN, DCF, RBJ, GPS e PD. Data analysis: JPA, RBN, DCF, RBJ, GPS e PD. Contribution with reagents / materials / analysis tools: JPA, RBN, DCF, RBJ, GPS e PD. Article Writing: JPA, RBN, DCF, RBJ, GPS e PD.

Conflicts of interest

The authors declare that they have no conflict of interest.

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5.7 LEGENDS TO THE FIGURES

Figure 1. Timeline of study design. Acute myocardial infarction, MI; animals submitted to the surgical procedure without occlusion from left coronary artery, Sham; resistance training, RT; continuous aerobic training, CAT; exercise tolerance test, ETT; 1st test of a maximum repetition, 1st 1RM; 2nd test of a maximum repetition, 2nd 1RM; 3rd test of a maximum repetition, 3rd 1RM; and 4th test of a maximum repetition, 4th 1RM.

Figure 2. Chart **A**: Number of series. Chart **B**: Number of repetitions. Values are mean ± SD, Sed-Sham, sedentary sham rats group (n = 8); RT-Sham, resistance training sham rats group (n=8); RT+CAT-Sham, resistance training combined with continuous aerobic training sham rats group (n=7); Sed-HF, sedentary heart failure rats group (n=6); RT-HF, resistance training heart failure rats group (n=6) and RT+CAT-HF, resistance training combined with continuous aerobic training heart failure rats group (n=6). *P<0.05 compared to RT-H and RT+CAT-HF groups. †P<0.05 compared to RT-HF group.

Figure 3. **A-D**: Maximum strength gain over time, absolute load. Maximum strength gain over time, relative load. Magnitude of absolute strength gain between the segments of the tests. Magnitude of the relative strength gains between the segments of the tests. Values are mean ± SD, Sed-Sham, sedentary sham rats group (n = 8);RT-Sham, resistance training sham rats group (n=8); RT+CAT-Sham, resistance training combined with continuous aerobic training sham rats group (n=7); Sed-HF, sedentary heart failure rats group (n=6); RT-HF, resistance training heart failure rats group (n=6) and RT+CAT-HF, resistance training combined with continuous aerobic training heart failure rats group (n=6). Graph **A** and **B**: *P<0.05 compared to the 1st 1RM for all training groups. †P<0.05 compared to sedentary groups. Graph **C** and **D**: *P<0.05 compared the percentage differences of the 2nd 1RM vs 3rd 1RM tests and the 3rd 1RM vs 4th 1RM tests for all training groups. †P<0.05 compared the percentage differences of the 2nd 1RM vs 3rd 1RM tests for the RT+CAT-HF group.

Figure 4. Exercise tolerance test (ETT). **A**: Speed (m/s); **B**: Time (s) and **C**: Distance (m). Values are means ± SD. Sed-Sham, sedentary sham rats group (n = 8); RT-Sham, resistance training sham rats group (n=8); RT+CAT-Sham, resistance training combined with continuous aerobic training sham rats group (n=7); Sed-HF, sedentary heart failure rats group

(n=6); RT-HF, resistance training heart failure rats group (n=6) and RT+CAT-HF, resistance training combined with continuous aerobic training heart failure rats group (n=6). *P<0.05 compared to the Sed-HF and RT-HF. $^{\dagger}P$<0.05 compared to all groups. $^{\ddagger}P$<0.05 compared to Sed-HF, RT-HF and Sed-Sham. $^{\S}P$<0.05 compared to Sed-HF and RT-HF. $^{\#}P$<0.05 compared to pre ETT. **P<0.05 compared to HF groups. $^{\S\S}P$<0.05 compared to Sed-HF, RT-HF, Sed-Sham and RT-Sham. $^{\&}P$<0.05 compared to Sed-HF.

Figure 5. Analysis of collagen volume fraction expressed by the percentage of interstitial collagen. Values are means ± SD. Sed-Sham, sedentary sham rats group (n = 8);RT-Sham, resistance training sham rats group (n=8); RT+CAT-Sham, resistance training combined with continuous aerobic training sham rats group (n=7); Sed-HF, sedentary heart failure rats group (n=6); RT-HF, resistance training heart failure rats group (n=6) and RT+CAT-HF, resistance training combined with continuous aerobic training heart failure rats group (n=6). *P<0.05 compared to all groups. $^{\dagger}P$<0.05 compared to Sham groups.

Figure 6. Analysis of inflammatory markers expressed as pg/ml. Values are means ± SD. Sed-Sham, sedentary sham rats group (n = 5); RT-Sham, resistance training sham rats group (n=7); RT+CAT-Sham, resistance training combined with continuous aerobic training sham rats group (n=6); Sed-HF, sedentary heart failure rats group (n=4); RT-HF, resistance training heart failure rats group (n=4) and RT+CAT-HF, resistance training combined with continuous aerobic training heart failure rats group (n=4).

Figure 7. Correlations between left ventricle end-diastolic pressure (LVEDP) with volumes of sets and repetitions. Correlation between volume of collagen fraction with volume of repetitions. Red circles (●), RT-HF group and black circles (●), RT+CAT-HF. **A:** LVEDP with volume of series; **B:** LVEDP with volume of repetitions and **C:** collagen volume fraction with volume of repetitions.

Table 1. Morphological variables and congestions

	Sed-Sham	RT-Sham	RT+CAT-Sham	Sed-HF	RT-HF	RT+CAT-HF
Initial weight, g	262.8±9.9	271.9±9.5	266.0±10.2	252.5±12.5	274.2±10.9	271.0±15.4
Final weight, g	375.4±17.4	363.1±33.8	350.1±32.6	351.5±38.9	350.8±32.6	360.8±11.8
Infarcted area%	----------	----------	----------	43.07±2.7	40.20±2.4	41.30±3.3
LVW/BW mg/g	2.41±0.2*	2.18±0.1*	2.56±0.2†	2.82±0.4	2.91±0.6	2.84±1.1
RVW/BW mg/g	0.57±0.1	0.61±0.1	0.54±0.1	1.20±0.3‡	0.72±0.3†	0.49±0.1
MW/BW mg/g	2.93±0.2	2.74±0.1	3.06±0.2	4.02±0.4§	3.66±0.1#	3.32±0.1**
Pulmonary congestion (PC) %	69.66±3.8	67.18±5.05	68.20±3.6†	79.06±1.6‡	69.22±3.4	72.62±1.01
Hepatic congestion (HC) %	70.58±0.7	69.58±0.7	69.40±1.0	70.97±1.7	70.06±0.6	69.68±0.9

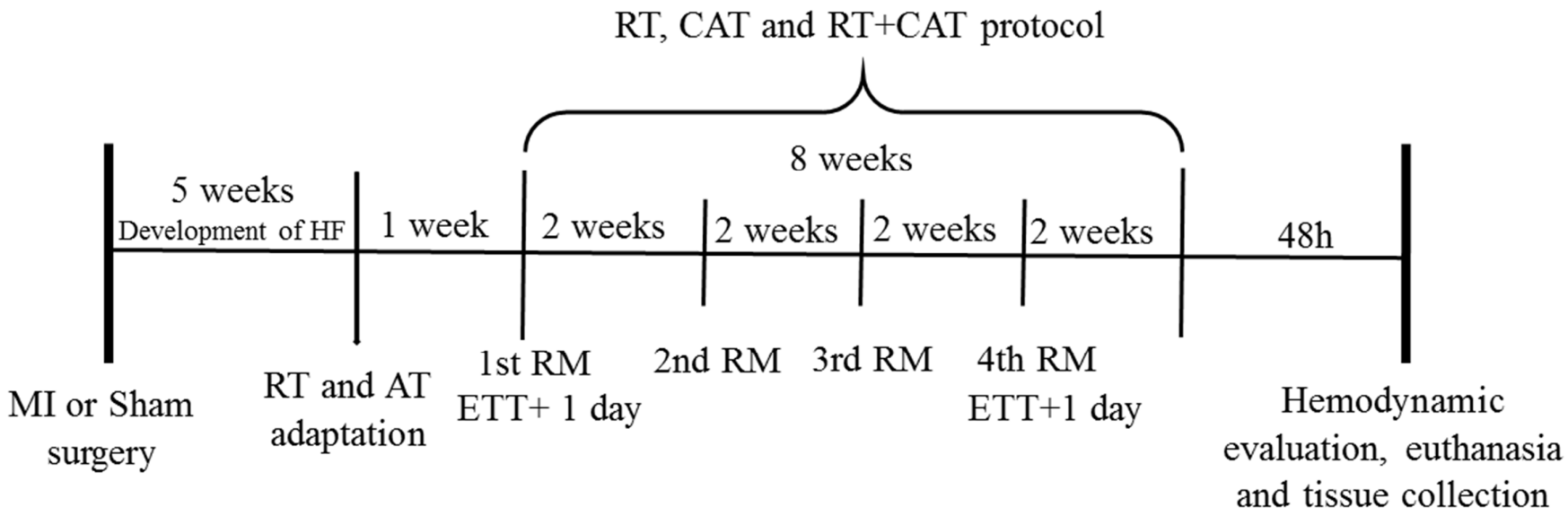
Values are means ± SD. Sed-Sham, sedentary sham rats group (n = 8);RT-Sham, resistance training sham rats group (n=8); RT+CAT-Sham, resistance training combined with continuous aerobic training sham rats group (n=7); Sed-HF, sedentary heart failure rats group (n=6); RT-HF, resistance training heart failure rats group (n=6) and RT+CAT-HF, resistance training combined with continuous aerobic training heart failure rats group (n=6) . Ratio of the total myocardial Weight to body weight, MW/BW; ratio of left ventricular weight to body weight, LVW/BW and ratio of right ventricle weight to body weight, RVW/BW. *P<0.05 compared to HF groups. †P<0.05 compared to RT+CAT-HF. ‡P<0.05 compared to all groups. §P<0.05 compared to sham groups and RT+CAT-HF. #P<0.05 compared to all Sham groups. **P<0.05 compared to RT-Sham.

Table 2. Hemodynamic parameters of the experimental groups.

	Sed-Sham	RT-Sham	RT+CAT-Sham	Sed-HF	RT-HF	RT+CAT-HF
HR, bpm	231.9±43.7	207.3±39.5	254.3±90.2	255.8±20.3	255.1±30.1	225.2±22.2
SBP, mmHg	104.7±23.3	111.7±17.7	108.9±17.3	94.86±16.1	109.8±20.2	118.3±03.1
DBP, mmHg	80.74±18.7	82.97±8.6	81.21±10.6	78.22±20.1	74.80±8.1	87.20±5.2
MAP, mmHg	93.29±20.0	97.92±11.8	96.07±14.1	85.85±8.9	87.46±12.5	97.63±7.2
LVEDP, mmHg	5.17±1.4	5.87±1.9	4.77±1.7	27.8±3.6*	9.8±2.9†	8.3±2.6
LVSP, mmHg	111.7±22.7	118.2±21.9	115.2±19.3	82.20±14.1‡	105.4±7.6	110.5±6.9
$+dP/dt_{max}$, mmHg/s	5846.8±1182	5929.8±962.6	6326.6±1553	3345.2±996.8 ‡	4647.9±804.7§	4889.9±501.8§
$-dP/dt_{max}$, mmHg/s	-4348.3±898.0	-4611.0±690.5	-5303.1±1021**	-2375.1±868.5†	-3387.7±325.9§	-3255.1±336.6§

Values are means ± SD. Sed-Sham, sedentary sham rats group (n = 8);RT-Sham, resistance training sham rats group (n=8); RT+CAT-Sham, resistance training combined with continuous aerobic training sham rats group (n=7); Sed-HF, sedentary heart failure rats group (n=6); RT-HF, resistance training heart failure rats group (n=6) and RT+CAT-HF, resistance training combined with continuous aerobic training heart failure rats group (n=6) . Heart rate, HR; Systolic blood pressure; SBP; diastolic blood pressure, DBP; Mean arterial pressure, MAP; end diastolic pressure of the left ventricle, LVEDP; left ventricular systolic pressure, LVSP; maximum positive left ventricular change in pressure over time, $+dP/dt_{max}$; Maximum negative left ventricular change in pressure over time, $-dP/dt_{max}$. $^{*}P<0.05$ compared to all groups. $^{\dagger}P<0.05$ compared to sham groups. $^{\ddagger}P<0.05$ compared to sham groups and RT+CAT-HF. $^{\S}P<0.05$ compared to RT-Sham and RT+CAT-Sham groups. ** $P<0.05$ compared to Sed-Sham.

Fig.1


RT, CAT and RT+CAT protocol
8 weeks
5 weeks
Development of HF
1 week
2 weeks
2 weeks
2 weeks
2 weeks
48h
MI or Sham surgery
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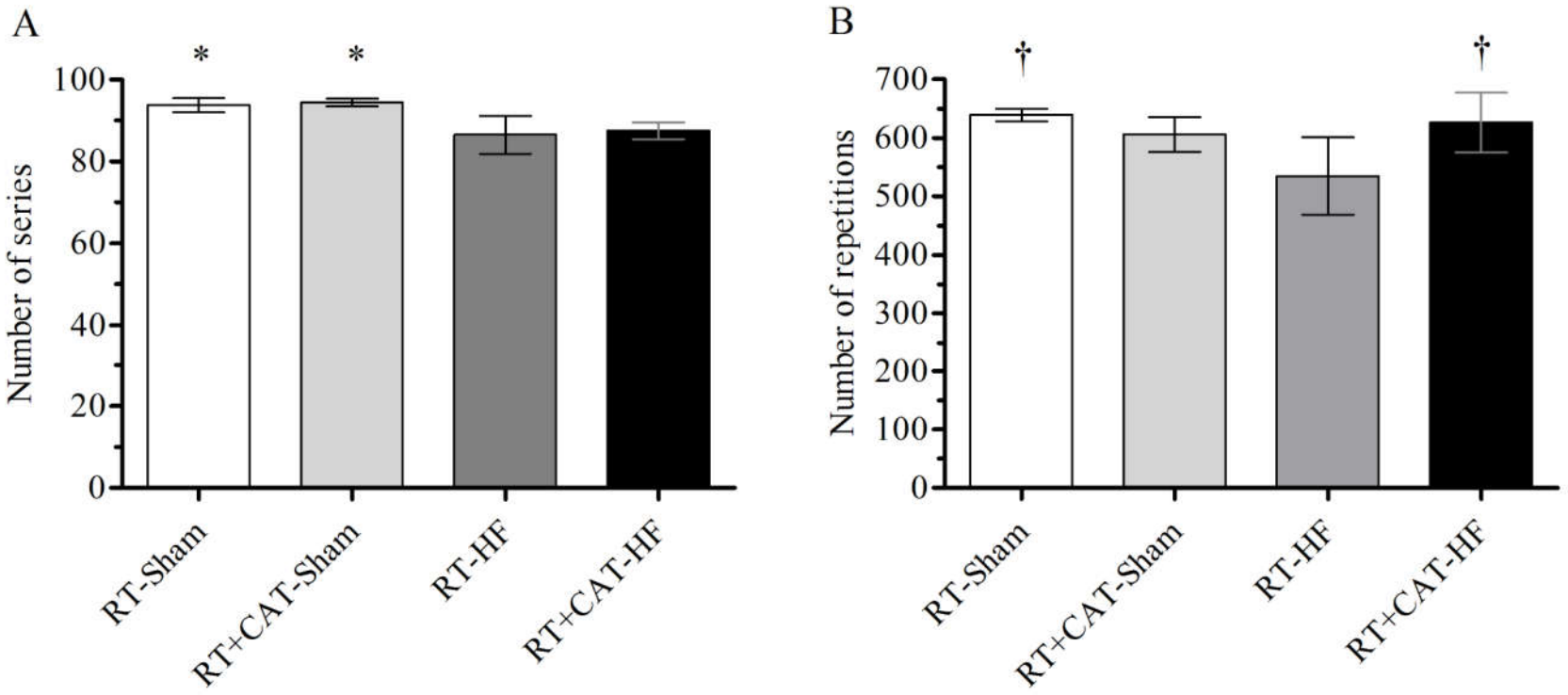
1st RM ETT+ 1 day
2nd RM
3rd RM
4th RM ETT+1 day
Hemodynamic evaluation, euthanasia and tissue collection

Fig. 2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RT-Sham
RT+CAT-Sham
RT-HF
RT+CAT-HF
B
Number of repetitions
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300
200
100
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RT-Sham
RT+CAT-Sham
RT-HF
RT+CAT-HF

Fig. 3

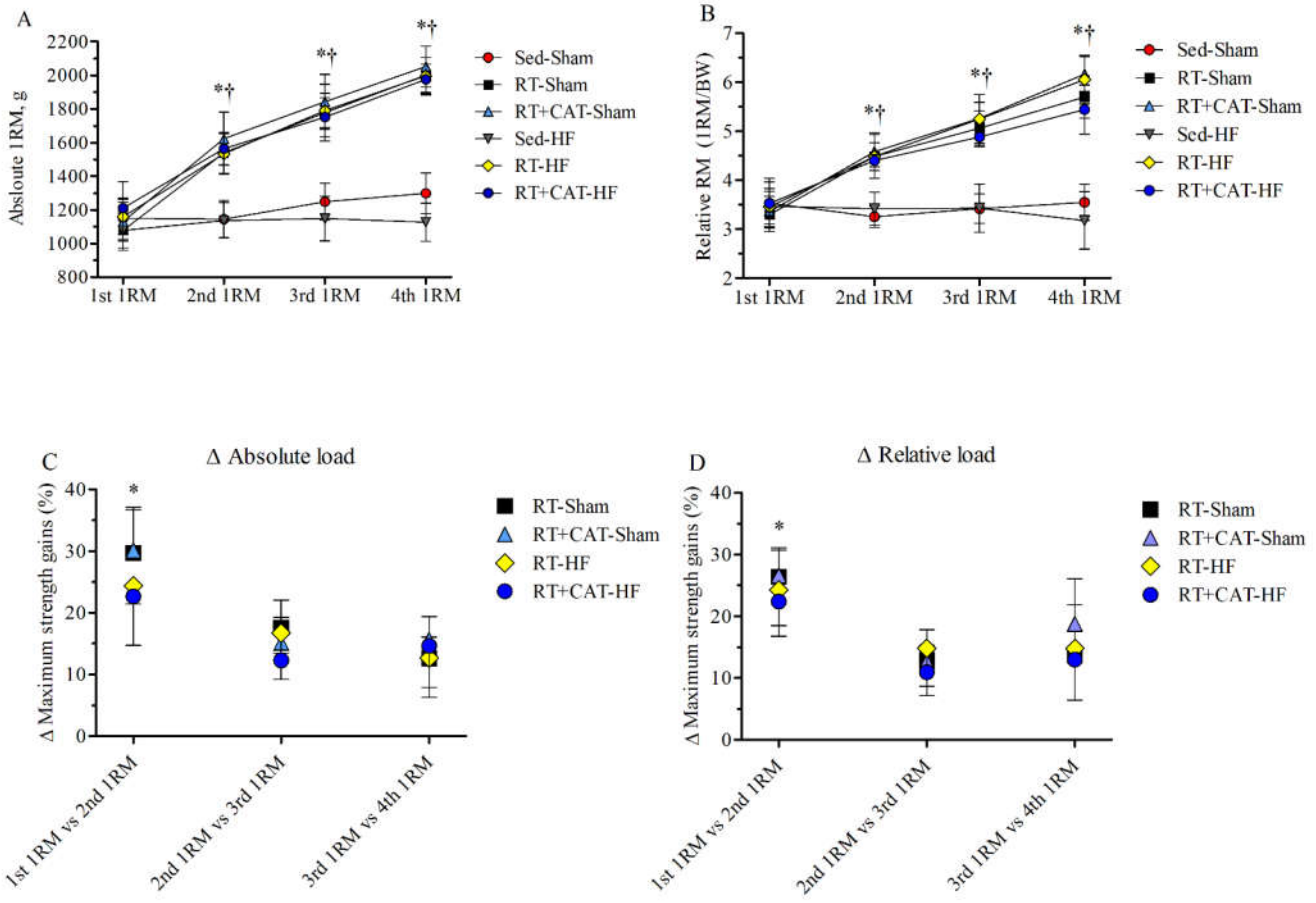
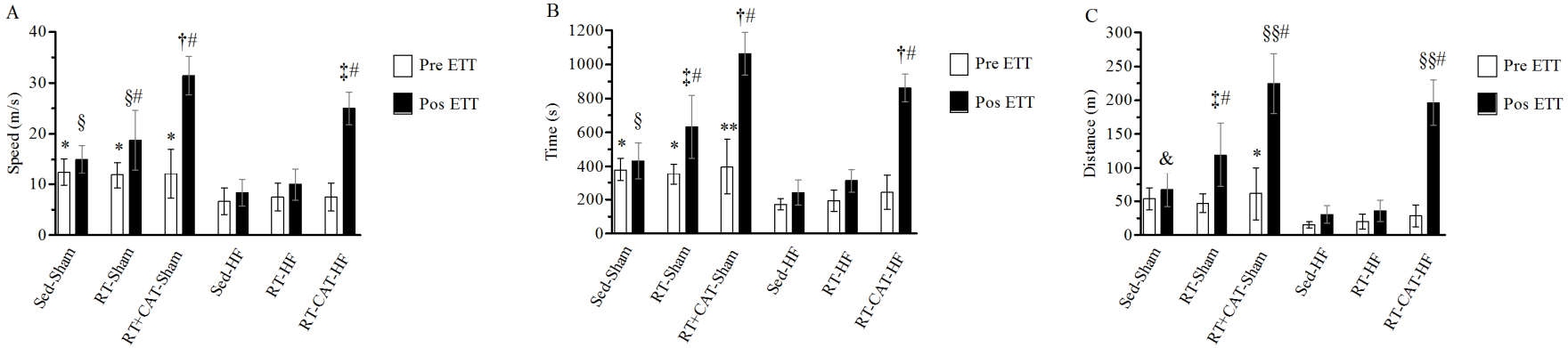

A
Absloute 1RM, g
2200
2000
1800
1600
1400
1200
1000
800
1st 1RM
2nd 1RM
3rd 1RM
4th 1RM
*†
Sed-Sham
RT-Sham
RT+CAT-Sham
Sed-HF
RT-HF
RT+CAT-HF
B
Relative RM (1RM/BW)
7
6
5
4
3
2
C
Δ Absolute load
Δ Maximum strength gains (%)
40
30
20
10
0
*
1st 1RM vs 2nd 1RM
2nd 1RM vs 3rd 1RM
3rd 1RM vs 4th 1RM
RT-Sham
RT+CAT-Sham
RT-HF
RT+CAT-HF
D
Δ Relative load

Fig. 4


A
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Pre ETT
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B
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RT+CAT-Sham
Sed-HF
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C
Distance (m)
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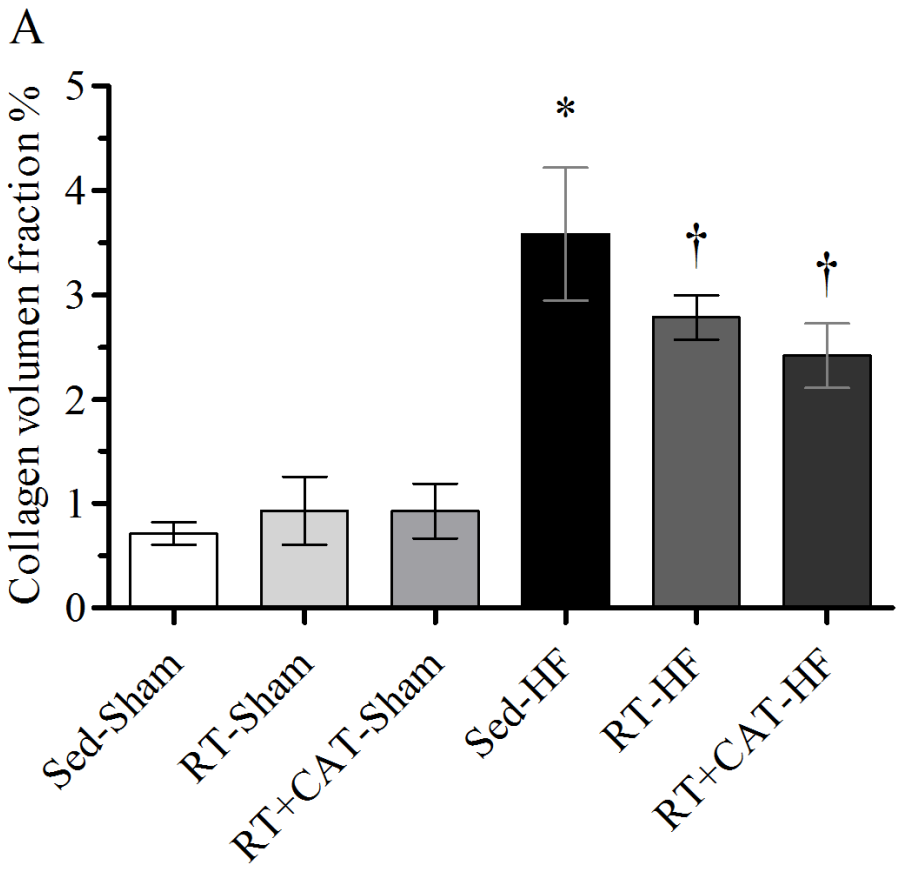
RT-Sham
RT+CAT-Sham
Sed-HF
RT-HF
RT-CAT-HF

Fig. 5


A
Collagen volumen fraction %
0
1
2
3
4
5
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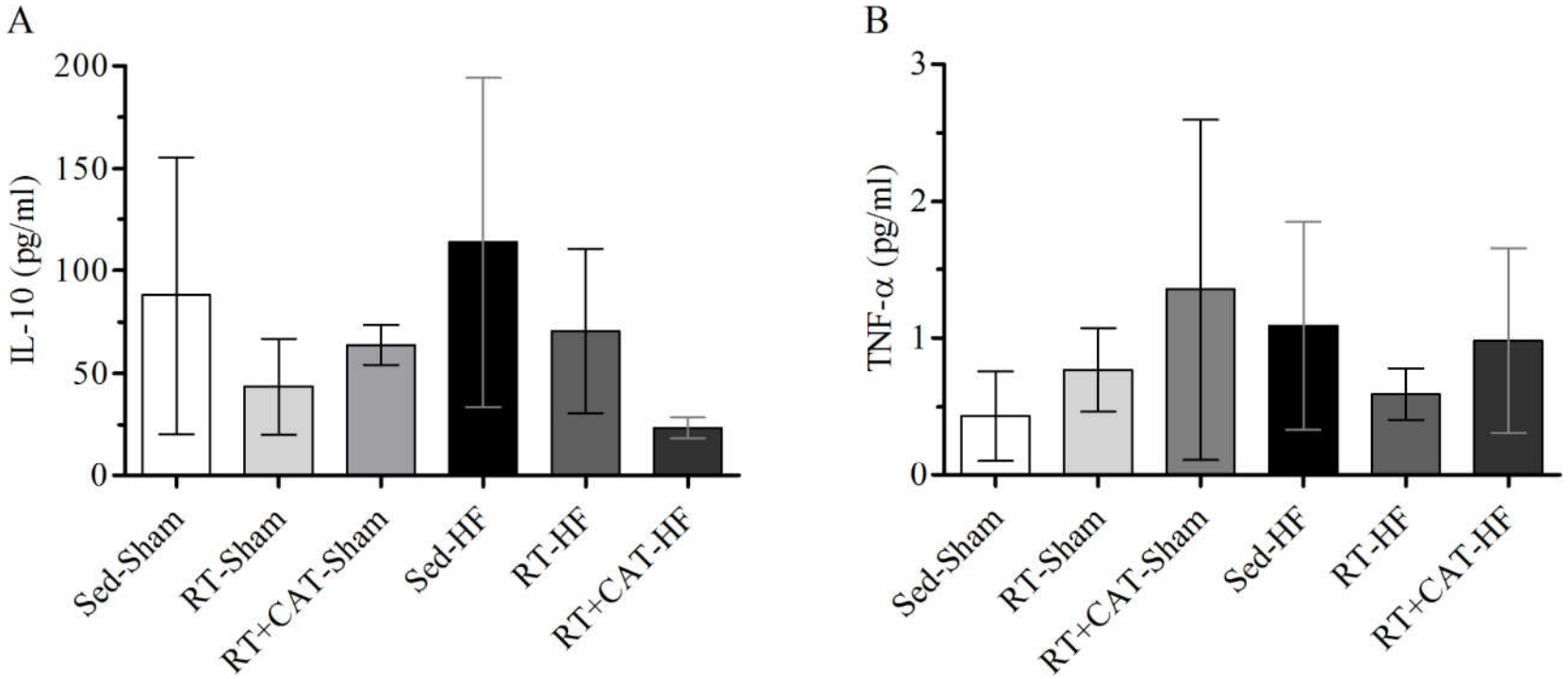
Sed-Sham
RT-Sham
RT+CAT-Sham
Sed-HF
RT-HF
RT+CAT-HF

Fig. 6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IL-10 (pg/ml)
200
150
100
50
0
Sed-Sham
RT-Sham
RT+CAT-Sham
Sed-HF
RT-HF
RT+CAT-HF
B
TNF-α (pg/ml)
3
2
1
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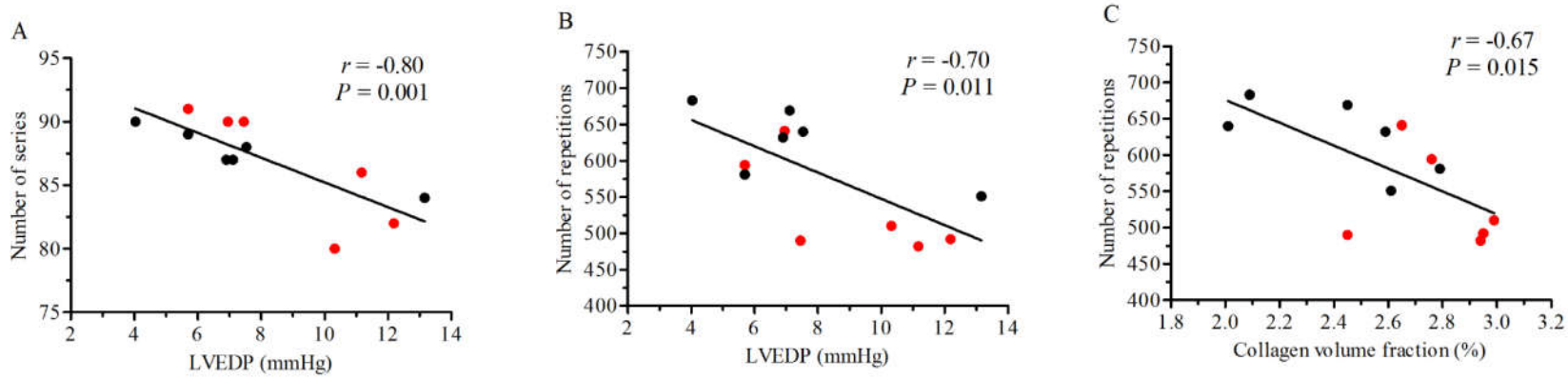
Sed-Sham
RT-Sham
RT+CAT-Sham
Sed-HF
RT-HF
RT+CAT-HF

Fig. 7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r = -0.80
P = 0.001
Number of series
LVEDP (mmHg)
B
r = -0.70
P = 0.011
Number of repetitions
LVEDP (mmHg)
C
r = -0.67
P = 0.015
Number of repetitions
Collagen volume fraction (%)

6. Conclusões

Os estudos realizados na presente tese foram planejados dentro de uma visão translacional, nosso intuito foi contribuir com novas estratégias na área do exercício do físico frente ao cenário fisiopatológico da IC. Dessa forma, a presente tese conclui que:

- ✓ O protocolo de TF de alta intensidade isolado ou associado com TA de moderada intensidade aumentou a força muscular dos animais com IC e essa resposta está correlacionada com a melhora da função ventricular;
- ✓ Os protocolos isolados ou combinados foram capazes de atenuar o volume da fração de colágeno, demonstrando que o TF de alta intensidade e o TA isolados ou combinados influenciam no remodelamento cardíaco de animais com IC;
- ✓ O TF isolado ou combinado com TA não foi capaz de modular positivamente a IL-10, pois apenas o TA isolado obteve respostas positivas nesse marcador anti-inflamatório, possivelmente a intensidade empregada e a frequência de treinamento (3X/semana) podem ter contribuido negativamente nesse modelo de IC;
- ✓ Animais com IC submetidos aos protocolos de TF isolado ou combinado com TA não apresentaram diferenças estatísticas no ganho de força e capacidade funcional comparados aos animais saudáveis, esses efeitos demonstram que a alta intensidade não é nociva para os animais com a síndrome complexa da IC;
- ✓ O volume de séries e repetições influenciaram de forma positiva na função cardíaca e no remodelamento ventricular de animais com IC submetidos aos protocolos de alta intensidade, esses achados demonstram que existe uma dose resposta do TF em relação ao tempo de exposição em animais com IC.

Adicionalmente, nossos resultados também demonstraram que os animais com IC e saudáveis têm a maior magnitude no ganho de força nas duas primeiras semanas de treinamento e nas semanas subsequentes os ganhos permanecem, mas em uma menor escala. Dessa forma, o modelo de agachamento para ratos e o

modelo experimental de IM são protocolos viáveis para testarmos novas estratégias, sejam elas isoladas ou combinadas com terapias, exercício físico ou fármacos.

Anexos

Anexo A - PARECER CONSUBSTANCIADO DE PROJETO DE PESQUISA E ENSINO

REPÚBLICA FEDERATIVA DO BRASIL
MINISTÉRIO DA EDUCAÇÃO

UFCSPA
UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

CEUA –COMISSÃO DE ÉTICA NO USO DE ANIMAIS

PARECER CONSUBSTANCIADO DE PROJETO DE PESQUISA E ENSINO

1) PROTOCOLO Nº: 102/12

2) DATA DO PARECER: 12/06/13 **Parecer 199/13**

3) TÍTULO DO PROJETO:

Efeitos do treinamento de força isolado e combinado ao treinamento aeróbico sobre a função hemodinâmica, remodelamento cardíaco e perfil bioquímico de ratos com insuficiência cardíaca.

4) PESQUISADOR RESPONSÁVEL:

Pedro Dal Lago

5) RESUMO DO PROJETO:

O projeto preconiza avaliação dos efeitos do treinamento força isolado e combinado ao treinamento aeróbico sobre a função hemodinâmica, remodelamento cardíaco e perfil bioquímico em ratos com insuficiência cardíaca induzida mediante infarto do miocárdio induzido cirurgicamente.

6) OBJETIVOS DO PROJETO:

Avaliar os impactos do treinamento de força isolado e combinado ao treinamento aeróbico sobre a função hemodinâmica, remodelamento cardíaco e perfil bioquímico em animais com insuficiência cardíaca.

7) FINALIDADE DO PROJETO: ☐ Ensino ☒ Pesquisa

8) ITENS METODOLÓGICOS E ÉTICOS DO PROJETO:

Título	[x]	Adequado	[ ]	Comentários
Introdução	[x]	Adequada	[ ]	Comentários
Objetivos	[x]	Adequados	[ ]	Comentários
Relevância e Justificativa	[x]	Adequados	[ ]	Comentários
Materiais e Métodos	[x]	Adequados	[ ]	Comentários
Cronograma para execução da pesquisa	[x]	Adequado	[ ]	Comentários
Orçamento e fonte financiadora	[x]	Adequados	[ ]	Comentários
Referências Bibliográficas	[x]	Adequadas	[ ]	Comentários

9) O PROJETO ESTÁ ADEQUADO À LEGISLAÇÃO VIGENTE:

[x] Sim [ ] Não

10) INFORMAÇÕES RELATIVAS AOS ANIMAIS:

Grau de dor/estresse: **B** [ ] **C** [ ] **D** [x] **E** [ ]

Justifique:

Espécie: *Rattus norvergicus* **Número Amostral:** 168

Redução Amostral: [ ] Sim [x] Não

Justifique:

Substituição de Metodologia: [ ] Sim [x] Não

Se achar necessário, justifique e sugira uma nova metodologia:

Aprimoramento da Metodologia: [ ] Sim [x] Não
Se achar necessário, justifique e sugira aprimoramentos da metodologia:

Acomodação e manutenção dos animais: [x] Adequada [ ] Inadequada
Se achar inadequada cite abaixo as melhorias necessárias:

Manipulação dos animais: [x] Adequada [ ] Inadequada
Se achar inadequada cite abaixo as melhorias necessárias:

Analgesia dos animais (se aplicável): [x] Adequada [ ] Inadequada
Se achar inadequada cite abaixo as melhorias necessárias com analgésico substituto:

Anestesia dos animais (se aplicável): [x] Adequada [ ] Inadequada
Se achar inadequada cite abaixo as melhorias necessárias com anestésico substituto:

Preencher adequadamente o Protocolo para Uso de Animais na Pesquisa.

Eutanásia dos animais (se aplicável): [x] Adequada [ ] Inadequada
Se achar inadequada cite abaixo as melhorias necessárias com metodologia substituta:

Local de Realização (Biotério/Labotarório): Laboratório de Fisiologia UFCSPA (Salas 10,12 e 14). Prédio Anexo II, Pós-Graduação.

11) CRONOGRAMA DE UTILIZAÇÃO DE ANIMAIS

Data	Espécie	Sexo	Quantidade
Nov/12 – Set/13 –	**Rattus norvergicus**	**Machos**	**42 animais**
Jul/13 – Mai/15	**Rattus norvergicus**	**Machos**	**42 animais**

12) RECOMENDAÇÃO:

[X] Aprovado

☐ Com Pendência

☐ Não aprovado

Comentários gerais sobre o projeto:

Entrar em contato com o pesquisador para conversar sobre a assinatura do outro membro do grupo

Anexo B – NORMAS DA REVISTA *JOURNAL OF APPLIED PHISIOLOGY*

Manuscript Formatting Requirements

Contents

File Formats for Online Submission and Publication

Manuscripts may be submitted to the **APS Peer Review system** in the following formats: Microsoft Word (.doc or .docx), Rich Text Format (.rtf), or PDF. Manuscript submissions should contain all required elements, such as the abstract, all main text, bibliography, figures, figure legends, tables, and table legends, and any proposed **supplemental material** in a single file.
APS accepts manuscripts in one of two formats: double-spaced in wide, one-column, traditional manuscript format, or single-spaced in two-column journal format. You may embed copies of the figures into the text for review purposes, but if you choose to embed figures, any revisions to figures during review (see instructions for **Preparing Figures**) will require you to upload the newly revised individual figure files to our online submission system, and the copies you had previously embedded must also be updated to reflect the revised figures.

Style

All text should conform to standard American English style and usage. Authors for whom English is not their native language are strongly encouraged to seek the aid of a professional English language editorial service. Be sure that the language in your manuscript is original, without inclusion of any previously published textual passages (including those from authors' own prior publications). Authors may wish to screen their manuscript for textual similarities prior to submission using fee for service scholarly publishing databases such as **iThenticate** or other free general screening databases including **Plagiarism Checker**. Please note that APS does not endorse any screening program nor guarantees that these screening tools will detect all instances of textual overlap.

The following companies specialize in life sciences and medicine (and other areas of science) and will edit your manuscript for a fee. Please note that these companies are not associated with the American Physiological Society.

- Cambridge Proofreading LTD
- Editage
- Editing/Proofreading
- International Science Editing
- ScienceDocs
- SciTechEdit International
- Scribendi
- Stallard Scientific Editing
- Write Science Right

Spelling and Editorial Style

Authors should consult *Webster's Third New International Dictionary or Merriam Webster's Collegiate Dictionary*, 11th edition, for spelling and compounding. The APS Journals follow American English rules for spelling. **All manuscripts will be edited by highly trained professional copy editors, according to the APS house style and guidelines.**

Abbreviations, Symbols, and Terminology

Abbreviations should be defined at first usage. However, at the discretion of the APS editorial staff, many internationally accepted (or otherwise compellingly conventional) abbreviations do not need to be defined; please consult the **list of accepted abbreviations**. For word usage, symbols, etc., authors are referred to *Scientific Style and Format: The CBE Manual for Authors, Editors, and*

Publishers(6th ed., 1994). For chemical and biochemical terms and abbreviations, consult the recommendations of the ***IUPAC-IUB Combined Commission on Biochemical Nomenclature*****.** Isotope specification must conform to the IUPAC system. Authors are referred to the following articles for style in specialized fields: **"Glossary on respiration and gas exchange" (*J Appl Physiol* 34: 549-558, 1973)**; and **"Glossary of terms for thermal physiology" (*J Appl Physiol* 35: 941-961, 1973)**.

Special Symbols

For special characters not available on the standard 104-key keyboard (e.g., Greek characters, mathematical symbols, figure symbols), use the Symbol font or use the "Insert Symbol" function in Microsoft Word; ***do not*** use Math font or image files (e.g., GIF) within the text for special characters or text constructions.

Trade Names

Proprietary (trademarked) names should be capitalized, with the spelling carefully checked. The generic name or generic descriptor accompany the trade name the first time it appears.

Cell Lines and Reagents

In the Methods section, the source of cells utilized (species, sex, strain, race, age of donor, whether primary or established) should be clearly indicated. The Methods section should state if cell line authentication has been carried out and by what method (e.g., STR profiling). The source of reagents should be stated (name, city, and state within parentheses) when first cited. If tests to rule out the presence of mycoplasmal contamination were not performed, this fact should be clearly stated. Other data relating to unique biological, biochemical, and/or immunological markers should also be included if available, with their source identified. Publication of results is based on the principle that results must be independently verifiable. Authors are expected to make unique reagents available to qualified investigators either directly or through a recognized distributor. See also**standards for cell line authentication**, **Unique Materials and Data Banks** **and** **Ethical Policies** for other requirements.

Manuscript Composition

Contents

Title Page

All submissions must contain a title page however brief the article may be (including, but not limited to, brief items such as editorials). The title page must contain the full title of the article; author(s) name(s); all departments and institutions in which the work was done; an abbreviated title for the running head; and the corresponding author's name, e-mail, and physical address for correspondence. Only one author may be designated as the corresponding author.

Title

Make the title succinct and informative. Avoid unnecessary words like "Studies in....". The title must not exceed 160 characters, including spaces between words.

Authors

List all authors' names and their first names or initials exactly as they should be known. Do not include any specific titles (e.g., PhD, MD, and Prof. are not needed). Include a brief itemized list of how each author contributed to the study.

"Group authorship" is allowed, with the name of a group (such as a consortium or program) to be listed as an author, with members of the group listed in the Acknowledgements section; however, the Program Director of the named group must be the one who signs for the group when the group's "author" signature is needed, i.e., on a Mandatory Submission Form or a Change of Authorship Form.

Authors who publish in APS journals may now present their names in non-Latin characters (in their native writing system) along side the standard English transliteration of their name in the main author line of the published article; for example, "Ta-Ming Wang (王大明)". We will accept any non-Latin languages that have standard **Unicode** characters designated for the native characters. For authors that choose this option, please only provide the native expression for the original written form of the transliterated name; that is, do not include any associated degree, rank, or title information in the native format. This feature is meant for the person's name only, not for ancillary information regarding academic achievement or institutional affiliation. To take advantage of this new feature, please insert the native expression of your name along side the English transliteration in the main title page of your manuscript submission.
See **Authorship Changes** for more information.

ORCID

The APS Journals program is now participating in the **ORCID Registry**. Authors submitting manuscripts to APS journals will now have the option to attach their ORCID identifier to their author profile in the APS Manuscript Submission system, or register for one if they are a new participant. This ORCID identifier will be included in the article metadata, if published, which may facilitate broad-reaching linkages of many pieces of an author's work across multiple databases (such Pubmed, PMC, and others).

ORCID is "an open, non-profit, community-based effort to create a permanent registry of unique researcher identifiers and a transparent method of linking research activities and outputs to these identifiers."

Please see the **ORCID website** for more information and to register for an ORCID identifier.

FundRef

The APS Journals program is now participating in the **FundRef Registry** (a CrossRef.org initiative).

Authors submitting manuscripts to APS journals will now be able to indicate the funder(s) associated with their work via an interface in the APS Manuscript Submission system. This FundRef identifier will be included in the article metadata, if published, which may facilitate discovery of the article by anyone who is able to search on that funder name in the FundRef Registry.

FundRef "provides a standard way to report funding sources for published scholarly research. Publishers deposit funding information from articles and other content using a standard taxonomy of funder names. This funding data is then made publicly available through CrossRef's search interfaces and APIs for funders and other interested parties to analyze."

Please see the **CrossRef** website for more information about the **FundRef Registry**.

Affiliation

List all departments and institutions in which the work was done, with city and state or country. Identify each author's affiliation by superscript numbers matched to the appropriate institution. Affiliation must reflect the organization(s) supporting the author(s) *while the research was done*. This may differ from the *current*affiliations of the author(s), which will be listed in such cases in the **Acknowledgments** section as the present address(es) of the author(s).

Running Head

The running head is an abbreviated version of the title, which will appear at the top of every page subsequent to the first page. Running heads must not exceed 60 characters including spaces between words.

Address for Correspondence

Only one author may be designated as the corresponding author. A full address for correspondence must be included, **with a current, valid e-mail address** for the corresponding author. The address of the corresponding author will appear on the first page of the article. Please note that a valid e-mail address is essential to participate in the APS electronic proofing service. Also,

provide your phone and fax numbers for use while your article is in production. If the contact information to be used during production differs from that to be included in the final article, indicate this explicitly.

Abstract

A one-paragraph abstract of not more than 250 words must accompany each manuscript. Longer abstracts may be subject to editorial truncation, to conform to the conventional perceptions of brevity that characterize an "abstract". The abstract should state what was done and why (including species and state of anesthesia), what was found (in terms of data, if space allows), and what was concluded. Even for short editorial-style articles, a brief "abstract" should be provided, if only to identify the topic (e.g., "This is an editorial summarizing recent new developments in physiology.").

New & Noteworthy

Participating Journals: ***AJP-Heart*** *(AHeart),* ***Journal of Applied Physiology****(JAPPL),* ***Journal of Neurophysiology*** *(JN),* ***AJP-Gastrointestinal*** *(AGI),* and ***Advances*** *(ADV)*.

New & Noteworthy is a brand of annotation summarizing what is new about an author's original research. Only authors submitting research articles to APS participating journals (see above) should submit their annotation in the online EJPress submission form field, ensuring that the exact statement also appears in their final submitted manuscript after Abstract and Keywords.

Specifications:

WORD LIMIT: 50 words for AHeart; 75 words for *JAPPL, JN, AGI,* and *ADV*.

AHeart, JAPPL, JN, AGI

- Annotations apply only to Original Research Articles, Innovative Methodologies, and Rapid Reports.
- Annotations appear in final published articles and the journal table of contents.
- Branded "New & Noteworthy"

ADV

- Annotations apply to all articles in the journal.
- Snapshot statement is solely posted on the journal's Facebook page with a link back to the published article.

Keywords

Include three to five words or short phrases relevant to the article.

Glossary

A glossary may be included (and is often helpful) in equation-laden articles with many different symbols (such as mathematical modeling or computational papers), specifying the units (and/or dimensions) as well as each definition. The glossary will usually precede the **Methods** section. See **this article** for an example.

Main Text

Introduction

Provide a brief overview of the scope and relevance of the study, especially with regard to previous advancements in related fields.

Materials and Methods

Describe techniques, cell/animal models used (including species, strain, and sex), and lists of reagents, chemicals, and equipment, as well as the names of manufacturers and suppliers, including URLS for those supplies obtained online, so that your study can be most easily replicated by others. For studies involving humans, the sex and/or gender of participants must be reported. Also in this section, describe the statistical methods that were used to evaluate the data. If clinical trials were used, a statement of registration is required; also, for all investigations involving humans or animals, a statement of protocol approval from an IRB or IACUC, or an equivalent statement, must be included (see **Guiding Principles for Research Involving Animals and Human Beings**). **All animal or human studies must contain an explicit statement that the protocols were submitted to, and approved by, an institutional review board or committee or that the protocols were performed under a license obtained from such a committee, board, or governing office.**

See **Experimental Details to Report in Your Manuscript** for more information.

See **Abbreviations, Symbols, and Terminology** for style information.

Results

Provide the experimental data and results as well as the particular statistical significance of the data.
See **Promoting Transparent Reporting of Results** for more information.
APS has published an **editorial** on the use of statistics, and authors are encouraged to consult it.

Discussion

Sometimes combined with the results in a section called "Results and Discussion". Explain your interpretation of the data, especially compared with previously published material cited in the References.

Appendix

An Appendix may be included (and is often helpful) in mathematical modeling or computational papers, e.g., to provide details of a solution strategy.

Acknowledgements

The acknowledgements section is where you may wish to thank people indirectly involved with the research (e.g., technical assistance; gifts of samples, reagents, or cell lines; loans of equipment or laboratory space; comments or suggestions during the creation of the manuscript). However, it is important that anyone listed here know in advance of your acknowledgement of their contribution, as documented during the submission process.

Current addresses of authors (which may differ from those in the affiliation line) may be included here.

Do not include "promissory notes." APS Journal policy is against inclusion of implicit or explicit promises that future work will be published.

Do not include dedications (e.g., to persons living or deceased). Dedications of articles are not permitted.

Grants

List the grants, fellowships, and donations that funded (partially or completely) the research. However, industry-sponsored grants should be listed under **Disclosures**.

Disclosures

Authors of research articles are required at the time of submission to disclose to the APS Publications Office any potential conflict of interest, financial or otherwise. See **Conflicts of Interest**. If the article is accepted for publication, information on the potential conflict of interest, or lack thereof, must be noted in the Disclosures section.

Footnotes

The footnotes section is the place to list any additional items pertinent to your article, including but not limited to **links to non-peer-reviewed data** that may be available to readers from your institutional web site.

Footnotes should be numbered consecutively throughout. They should be assembled on a separate page at the end of the article.

References

Authors are responsible for accuracy of citations. References must be limited to directly pertinent **published** works or papers that have been **accepted for publication**. An abstract, properly identified as "Abstract", may be cited only when it is the sole source.

Reference lists should be arranged alphabetically by author and numbered serially. The reference number should be placed in parentheses at the appropriate place in the text.

The style of citation should be as follows, with journal name abbreviated as in Medline, PubMed, and Index Medicus. Appropriate templates for your citation management software are available from the respective company websites (e.g., **EndNote**, **Reference Manager**).

The examples given below are shown with numbers because that is the style for most APS Journals, except for the *Journal of Neurophysiology* (see note, below, after these examples). The first is a standard book reference; the second is a standard reference to an "early view" or "prepress"

reference, such as the APS "Articles in PresS" (note the use of the “digital object identifier”—doi—in this citation); the third is a standard journal reference.

1. **Pollock DM.** Endothelin receptor subtypes and tissue distribution. In: *Endothelin Molecular Biology, Physiology, and Pathology*, edited by Highsmith RF. Totowa, NJ: Humana, 1998.

2. **Scarafia LE, Winter A, Swinney DC.** Quantitative expression analysis of the cellular specificity of HECT-domain ubiquitin E3 ligases. *Physiol Genomics* (April 26, 2001). doi:10.1152/physiolgenomics.00075.2001.

3. **Villalobos AR, Parmelee JT, Renfro JL.** Choline uptake across the ventricular membrane of neonate rat choroid plexus. *Am J Physiol Cell Physiol* 276: C1288-C1296, 1999.

MORE Example References

References for the *Journal of Neurophysiology* should be arranged alphabetically by author. The appropriate author name and year for each reference should be included in parentheses at the proper point in the text using the following style **(this is ONLY for the *Journal of Neurophysiology*, NOT for other APS Journals)**:

- one author (Brown 1982)
- two authors (Brown and Smith 1982)
- three or more authors (Brown et al. 1982).

For the in text citations in the *Journal of Neurophysiology*, here are some other important details. If more than two references are cited by different authors, separate entries with a semicolon (Brown 1982; Smith 1983). If more than two references are cited by the same first author (or single author), use "et al." where appropriate plus the date, even if the subsequent authors are not the same in all the references (Brown et al. 1982, 1983). Note the use of commas between two consecutive years or nonconsecutive years. Do **not** use dashes for ranges (Brown et al. 1982, 1983, 1986, 1987, 1988). If more than two references with the same year and author(s) are cited, use lowercase letters after the year (Brown 1982a, 1982b). Lowercase letters should be inserted in same-year references in the reference list.

Citing Unpublished Observations, Personal Communications, and "In Press" Manuscripts

Submitted papers still in preparation or in peer review and/or any other unpublished materials, observations, or personal communications cannot be included in the reference list, which may only list published work. However, such material can be cited in the text, but at submission, authors will be required to confirm that all individuals acknowledged in the manuscript are aware that they are being acknowledged and approve of the manner and the context of the acknowledgement. This includes, but is not limited to the following circumstances:

- to publish information disclosed in a personal communication or unpublished observation;
- to recognize additional individuals who helped in preparation of the manuscript;
- for permission from a copyright holder to discuss information that has been accepted for publication but is "in press" and not yet available, online or otherwise.

For both unpublished observations and personal communications, provide the cited person's last name and all initials.

Figure Captions

Every figure must have a descriptive figure caption, to describe to the reader in sentence form the relevant details of the figure, to place it in the proper context of the manuscript. Information reported in figures and legends should describe each individual experiment presented, including the number of samples or animals used per treatment. The statistics performed for each experiment should be reported as well. These textual figure captions must be listed in order in the manuscript, following the reference list.

Tables

Whenever possible, authors are encouraged to submit figures rather than tables. Statistical summary tables should be submitted when possible, rather than tables with many lines of individual values. Lengthy tables of data that cannot be presented in a suitable manner, according to APS standards of print publication, may be extracted and set as a supplement to the online article. These supplements remain an integral part of the article for the reader, as text referring to these tables will remain in the

article, and links directly to the supplements will be embedded and prominently indicated at all points of entry to the online article (see**Data Supplements**).

Submitted tables should adhere to the following guidelines:

- Tables must not duplicate material in text or figures.
- Tables should be numbered consecutively with Arabic numerals and prepared with the size of the journal page in mind: 3.5 in. wide, single column; 7 in. wide, double column.
- Each table should have a brief title; explanatory notes should be in the legend, not in the title.
- Nonsignificant decimal places in tabular data should be omitted.
- Short or abbreviated column heads should be used and explained if necessary in the legend.
- Statistical measures of variations, SD, SE, etc., must be identified. (Example: "Values are means ± SE.")
- Table footnotes should be listed in order of their appearance and identified by standard symbols: *, †, ‡, § for four or fewer; for five or more, consecutive superscript lowercase letters should be used (e.g., [a], [b], [c], etc.).

Equations

Manuscripts with many mathematical characters and equations should be prepared using **MathType** version 6.0 or higher (available from **Design Science, Inc.**). Manuscripts with a minimal amount of math may be prepared using word-processing tools such as Microsoft Word Equation Editor, or with features such as bold, italics, superscript and subscript together with characters in the Symbol or Greek fonts. Do not use the Wingdings or Webdings fonts. Please do not submit equations as images. Inline math text that can be regular text (e.g., x^2[n 2]) should be provided as such. Please do not set these as MathType.

Mathematical equations should be simplified as much as possible and carefully checked.

- Use the slant line (/) for simple fractions ($a + b$)/($x + y$) in the text rather than the built-up fraction $a + b$[over]$x + y$, which should only be used if the equation is offset from the text.
- Use subscripts or superscripts wherever feasible and appropriate to simplify the equations.
- Please use notation that is consistent with the standard nomenclature in applied mathematics. As an aid to the reader, please state the convention that you are following, especially if it is uncommon.
- Symbols should be defined as they first appear in the text. A **glossary**may be included (and is often helpful) in articles with many different symbols, specifying the units (dimensions) as well as each definition. The Glossary will usually precede the **Methods** section.
- APS style allows punctuation in displayed equations.

Mathematical Models

Presentation of the model(s) must be sufficiently clear to allow physiologists with limited experience in modeling to follow the model development, limitations, and physiological relevance. Assumptions concerning the importance of physiological processes included in the model should be clearly stated.

- If the model equation(s) require solution, the method of solution should be described in sufficient detail to permit readers to duplicate the solution in their own laboratories. Algorithms from commercial software libraries should be so identified. Details of the solution strategy may be summarized in an **Appendix**.
- For simulations, sources or estimation methods for all parameter values should be presented and the numerical values given in the text or a table. A sensitivity analysis must be performed for important parameters (covering ranges of values relevant to the manuscript) to determine how the model predictions are affected by numerical parameter values.
- If the model is used to estimate parameter values, measures of the uncertainties associated with the estimated parameter values should be presented.
- For models intended for use in a predictive setting, validation of the model with a data set not used for model parameter estimation (i.e., cross-validation) is recommended. Sensitivity analysis or parameter uncertainty determination is an important component of modern modeling practice that allows assessment of the validity of a model.
- Results obtained with the model(s) should be compared with appropriate physiological data, either from literature or from new experiments. Simulation results may be examined for prediction of changes or trends in physiological variables similar to those reported for in vitro or in vivo studies. The discussion should include information on the physiological significance of the model study, limitations of the model, and suggestions for new modeling and/or experimental studies.

Example References

Journal Articles

1. **Villalobos AR, Parmelee JT, Renfro JL.** Choline uptake across the ventricular membrane of neonate rat choroid plexus. *Am J Physiol Cell Physiol* 276: C1288-C1296, 1999.

Book References

1. **Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K.** *Current Protocols in Molecular Biology*. New York: Wiley, 1995, p. 25-26.

2. **Pollock DM.** Endothelin receptor subtypes and tissue distribution. In: *Endothelin Molecular Biology, Physiology, and Pathology*, edited by Highsmith RF. Totowa, NJ: Humana, 1998.

APS Handbook of Physiology Series

Large text books require very specific citation information. For example, the APS *Handbooks* series contains a huge amount of information, and the inclusion in the citation of the section, volume, part, and chapter is essential to aid the reader in finding the information quickly (please note that the APS chooses not to list editors for the APS *Handbooks*).

1. **Kaufman MP, Forster HV.** Reflexes controlling circulatory, ventilatory, and airway responses to exercise. In: *Handbook of Physiology. Exercise: Regulation and Integration of Multiple Systems. Control of Respiratory and Cardiovascular Systems.* Bethesda, MD: Am. Physiol. Soc., 1996, sect. 12, pt. II, chapt. 10, p. 381-447.

Articles Published on the Web

Many more reports than in past years are being published primarily, if not exclusively, on the World Wide Web. Such articles should be cited in the "online" style as shown below.

Author/editor (if known). (Revision or copyright date, if available). Title of page [Publication medium]. Page publisher. URL (Protocol://Site/Path/File) [Access date].

1. **Dudoit S, Yang YH, Callow MJ, Speed TJ.** Statistical methods for identifying differentially expressed genes in replicated cDNA microarray experiments [Online]. Dept. of Statistics, Univ. of California at Berkeley. http://www.stat.berkeley.edu/users/terry/zarray/Html/matt.html [3 Sept. 2000].

2. **Friedman N, Linial M, Nachman I, Peter D.** Using Bayesian networks to analyze expression data [Online]. Stanford University. http://robotics.stanford.edu/people/nir/Abstracts/FLNP1Full.html [2000].

Note that the date may be general or specific, to the day.

Some citations may have portions published in print and other relevant portions deposited online. However, if directions to the online portions are available in the printed work, this sort of citation should be avoided.

1. **Joachims T.** Making large-scale SVM learning practical. In: *Advances in Kernel Methods: Support Vector Learning,* edited by Schulkopf B, Burges C, and Smola A. MIT Press, 1999. [The software is available at http://www-ai.cs.uni-dortmund.de/SOFTWARE/SVM_LIGHT/svm_light.eng.html]

2. **Spellman PT, Sherlock G, Zhang MQ, Iyer VR, Anders K, Eisen MB, Brown PO, Botstein D, Futcher B.** Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. *Mol Biol Cell* 9: 3273-3297, 1998. [The data are available at http://cellcycle-www.stanford.edu]

DOIs and Early Publication in Articles in PresS

Current technology allows publication of an article in several editions. For example, the final, citable draft of an accepted article may be posted to a web site, pending final copyediting and page layout/design. This initial post to the web qualifies as publication, but eventually the article will reach the readership in a final, polished form.

The APS publishes peer-reviewed articles upon acceptance as Articles in PresS. These articles may be cited and establish publication's priority before they appear in final print and online forms. (Please note the required use of a "digital object identifier" -- DOI -- in this citation.)

1. **Scarafia LE, Winter A, Swinney DC.** Quantitative expression analysis of the cellular specificity of HECT-domain ubiquitin E3 ligases. *Physiol Genomics* (April 26, 2001). doi:10.1152/physiolgenomics.00075.2001.

However, once this article has reached its final stage of publication, it will be cited with is new publication data, as follows:

1. **Scarafia LE, Winter A, Swinney DC.** Quantitative expression analysis of the cellular specificity of HECT-domain ubiquitin E3 ligases. *Physiol Genomics* 4: 147-153, 2001. First published April 26, 2001; doi:10.1152/physiolgenomics.00075.2001.

Technical Documents, Congress Proceedings, etc.

Technical documents, congress proceedings, and some other sorts of material may often be published by the specific institution that sponsored the research.

1. **Efron R, Tibshirani B, Goss V, Chu G.** *Microarrays and Their Use in a Comparative Experiment* (Technical Report). Palo Alto, CA: Department of Statistics, Stanford University, 2000.

Corrigenda/Errata

If an article required a correction, after first publication, this should be noted in the citation of the original article.

1. **Hallows KR, Packman CH, Knauf PA.** Acute cell volume changes in anisotonic media affect F-actin content of HL-60 cells. *Am J Physiol Cell Physiol* 261: C1154-C1161, 1991. (Corrigendum. *Am J Physiol Cell Physiol*268: March 1995, following table of contents)

2. **Steenman M, Chen YW, Le Cunff M, Lamirault G, Varro A, Hoffman E, Leger JJ.** Transcriptomal analysis of failing and nonfailing human hearts. *Physiol Genomics* 12: 97-112, 2003. First published November 12, 2002; doi:10.1152/physiolgenomics.00148.2002. (Corrigendum. *Physiol Genomics*18:128, 2004)

Translations

1. **Fritsch G, Hitzig E.** Uber die Elektrische. In: *Some Papers on the Cerebral Cortex*, translated and edited by von Bomin G. Springfield, IL: Thomas, 1960, p. 73-96.

2. **Gavrilov KA, Perel TS.** Earthworms and other invertebrates in the soil under...[in Russian]. *Pochovedenia* 8: 133-140, 1958.

3. **Gavrilov KA, Perel TS.** Earthworms and other invertebrates in the soil under...[in Russian, English summary]. *Pochovedenia* 8: 133-140, 1958.

4. **Vol'Kensktein MV**. Molecules and Life: An Introduction to Molecular Biology. New York: Plenum, 1970. [Transl. from the Russian.]

Many Authors

It is APS Publications policy to list *all* authors in a research group. That is, the use of only the first author's name, followed by "et al." is unacceptable.

1. **Lander ES et al.** Initial sequencing and analysis of the human genome. *Nature* 409: 860-921, 2001.

should read:

1. **Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, LeVine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann N, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Grafham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Shownkeen R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissoe SL, Wendl MC, Delehaunty KD, Miner TL, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson P, Wenning S, Slezak T, Doggett N, Cheng JF, Olsen A, Lucas S, Elkin C, Uberbacher E, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissenbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubenfield M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM, Schmutz J, Dickson M, Grimwood J, Cox DR, Olson MV, Kaul R, Raymond C,**

Shimizu N, Kawasaki K, Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramser J, Lehrach H, Reinhardt R, McCombie WR, de la Bastide M, Dedhia N, Blocker H, Hornischer K, Nordsiek G, Agarwala R, Aravind L, Bailey JA, Bateman A, Batzoglou S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M, Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert JG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kaspryzk A, Kennedy S, Kent WJ, Kitts P, Koonin EV, Korf I, Kulp D, Lancet D, Lowe TM, McLysaght A, Mikkelsen T, Moran JV, Mulder N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowski J, Thierry-Mieg D, Thierry-Mieg J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YI, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, Szustakowki J, de Jong P, Catanese JJ, Osoegawa K, Shizuya H, Choi S, Chen YJ. Initial sequencing and analysis of the human genome. *Nature* 409: 860-921, 2001.

However, if there is an inclusive name for the research group as a whole (as in the case above, the " International Human Genome Sequencing Consortium"), it should be used rather than listing hundreds of authors.

So, for example, it is correct to cite:

1. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* 409: 860 921, 2001.

Preparing Figures

Figure preparation

Graphic File Formats

We recommend using Adobe Photoshop to crop and size photographic elements and Adobe Illustrator to prepare and assemble final figures for publication.

EPS and TIFF are the industry-standard file formats required for publication. Avoid, when possible, using MS PowerPoint to prepare your figures. JPEG, GIF, PNG, Excel, and Word files, etc. are not recommended formats for preparing your figures.

If you are not using an industry-standard graphics application, (e.g. Adobe Illustrator/Photoshop) then PDF is the recommended file format. **PDF files must be generated from the original figure at a high-quality setting** from the originating application. The PDF file format maintains design integrity to ensure what you send is what is received as it eliminates most instances of software and hardware incompatibility issues between Authors and APS.

Information about these program types, and other imaging software, can be found by clicking on the following links:

- **PowerPoint**
- **Photoshop**
- **Illustrator**

Figure Size

Figures should be generated at the size they are to appear in the journal (printed 1:1), when possible. Figures should be sized according to the table below. Please arrange elements and type to leave the least amount of white space. Figure panels should be clearly labeled with boldface uppercase letters (A, B, C, etc.). Figures submitted outside these specifications will be sized at the APS Art Department's discretion.

No. of Columns	Inches	Centimeters	Picas
Single	≤ 3.5	≤ 8.9	≤ 21
Double (side legend)	4 – 5	10 – 12	25 - 30
Full Width	6 – 7.15	15 - 18	36 - 43
Maximum depth	9	22.8	54

Font Size

Use Arial, Helvetica, Times or Times New Roman and Symbol only, if possible. This reduces the number of dropped characters / missing symbols. Choose one font and use it consistently throughout figures. Keep font size between 5-14 points.

Resolution

NOTE: Resolution requirements only apply to pixel-based (raster) images (i.e. TIFF, JPEG). These are most often photographic captures. They do not apply to vector-based images (i.e. EPS, AI, PPT, PDF) UNLESS they also contain raster images.

Always prepare photographic elements (raster-based) at print publication-quality resolution.

- Color and grayscale photographs ONLY: 300 dpi
- Color and grayscale photographs with text or line art/graphic elements: 600 dpi
- Line Art: 600 – 1200 dpi

Important: You must not increase the resolution of a raster image once it is set. Attempting to increase the resolution will artificially add "data" and "noise" to the image which will decrease the quality further.

Use of color for data presentation

Color is considered "scientifically necessary" when there is no other way to show the data in the figure. The use of color is not always scientifically necessary when showing graphic representations of data. Please submit in color only if you intend for the figure(s) to be published in color. **All color work submitted to APS must be saved as a RGB file (not CMYK).**Final decisions regarding the necessity of color use will be made by the Scientific Editor of the individual journal in conjunction with APS Publications.

Color figures are subsidized by APS at a cost to authors of $200 per figure, assuming that color is scientifically warranted and page charges are paid. **Scientifically unnecessary color figures are not permitted in the Journals**, and in such cases authors will be required to provide a black & white version suitable for print publication.

Examples of scientifically necessary vs. unnecessary color.
If the first or last author is an APS member in good standing, a special discount for color charges may apply (see Cost of Color), color figures that are scientifically necessary are published free of charge. Membership is not a criterion for publishing a figure in color.
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Still have questions about preparing your figures? Contact the APS **Art Department**.

Data Presentation

Electrophoretic gels, blots, and other digital images

Technology has made, and continues to make, the publication process faster, more accessible and robust. Technology and applications, such as Adobe Photoshop, also have made it easier to manipulate data. All accepted materials are evaluated for compliance with **APS policies** on image assembly and presentation.

Each gel or blot should contain a molecular-weight size marker. Each panel should retain space above and below the band of interest from the original image. It is not appropriate to crop the panel right on the band itself. All micrographs should include a scale bar. Moreover, the submitted images should faithfully represent those originally-captured. Therefore:

- **Authors should not** move, remove, introduce, obscure or enhance any specific feature within an image.
- **Authors should not** adjust contrast, color balance or brightness unless applied to the entire figure. However, these adjustments must not obscure, eliminate or misrepresent the originally-captured information. Any non-linear adjustment should be disclosed in the legend.
- **Authors should not** quantitatively compare samples from different gels/blots; if this is unavoidable authors must state in the legend that all samples were derived at the same time and processed in parallel.
- If gels, blots, or fields are grouped or rearranged, then authors must insert spaces or dividing lines to indicate these changes and disclose the arrangement in the figure legend.
- Where scientifically necessary, appropriate loading controls specific for the experiment presented should be inserted into submitted figures. If the experimental design means that the same loading control is appropriate for more than one analyte presented in one or more figures, ordinarily it is not necessary, or desirable, to present the loading control in every panel. If an image of the same loading control must be repeated in multiple panels, this must be disclosed in the relevant legend(s).

These policies are adapted from *The Journal of Cell Biology* (Instructions to Authors, 2011) and *Nature* (Online Instructions to Authors, 2011).

Any question of improper manipulation that is raised during the review or editing process will be brought to the Editor and Publications Committee Chair for review. If called into question, authors should be prepared to provide the following information:

- Original, unedited data/captures
- Acquisition software
- Information on image-processing software, as well as details regarding the operations performed

Acceptable Use of Photographs

Use of photographs to demonstrate experimental staging, tools, and apparatus in an experimental procedure should be kept to a minimum.

Photographs should show only the portion of the animal relevant to communicate the orientation of the body for the procedure.

Drawings and diagrams representing experimental procedures should be used in place of photographs whenever possible.

Photographs should avoid showing people handling animals, although gloved hands in the perimeter of an image may be allowed in close-up photographs.

Experimental Details to Report in Your Manuscript

Experimental Details to Report in Your Manuscript

Publication of research articles is based on the principle that results must be independently verifiable. Therefore, authors are expected to 1) describe the experiments performed and present the results as accurately as possible, 2) make unique reagents and resources available to qualified investigators either directly or through a recognized distributor and 3) store all data that are reported in the manuscript either on a secured server at their research institution or in a public repository. For more information on APS's efforts to enhance experimental reproducibility, see **Promoting Transparent Reporting in APS Publications to Enhance Data Reproducibility**.
Descriptions of the following materials are encouraged to include:

Cell Lines

- Source of cells utilized (species, sex, strain, race, age of donor, whether primary or established)
- Declaration of whether cell line authentication was performed and by what method (e.g., STR profiling)
- Declaration of whether testing for mycoplasma contamination was performed and the results of the tests

Reagents and Unique Materials

- Source of reagents and unique materials (Company name, distributer, developer, etc.)
- Data that confirms the specificity of the unique biological, biochemical, and/or immunological markers, submitted as "Supporting Data." If the data already exists, please reference the published article.

Antibodies

- Source of antibodies (company name, distributer, developer, etc.)
- One representative full blot detailing the validation of the each antibody used in the study for protein specificity, excluding loading controls.

- Lanes on the blot should be labeled to note the nonspecific and specific bands and the positive and negative controls. Exposure time should be indicated.
- Validation data should be submitted as **Supplemental Data.** If the data already exists, please reference the published article.

Description of the following studies should include:

Animal Studies

- An explicit statement that the animal use protocol was submitted to, and approved by an institutional committee or that the protocol was performed under a license obtained from such a committee, board, or governing office
- Authors are encouraged to refer to the **ARRIVE Guidelines Checklist** in preparation for reporting the methods and results of animal studies. Particular attention should be given to providing detailed information regarding:
  - Precise details of all experimental procedures (drug formulation and dose, anesthesia and analgesia used, method of euthanasia, etc.)
  - Steps taken to minimize subjective bias in the study design (randomization, blinding, etc.)
  - The animals used in the study (species, strain, sex, age, source of animals, genetic modification status, housing, diet, etc.)
  - The controls used in the study (littermate, purchased, identical conditions, contemporaneous, historical, etc.)

Human Studies

- An explicit statement that the protocol was submitted to, and approved by, an institutional review board or the protocol was performed under a license obtained from such a committee, board, or governing office
- The sex and/or gender of participants
- If the study is a clinical trial, a statement of registration is required

Information for the following data and analyses should include:

Electrophoretic gels, blots, and other digital images

- Each gel or blot should contain a molecular-weight size marker
- Each panel should retain space above and below the band of interest from the original image
- All micrographs should include a scale bar
- Images presented in the manuscript should faithfully represent those originally-captured:
  - **Authors should not** move, remove, introduce, obscure or enhance any specific feature within an image.
  - **Authors should not** adjust contrast, color balance or brightness unless applied to the entire figure. However, these adjustments must not obscure, eliminate or misrepresent the originally captured information. Any non-linear adjustment should be disclosed in the legend.
  - **Authors should not** quantitatively compare samples from different gels/blots; if this is unavoidable authors must state in the legend that all samples were derived at the same time and processed in parallel.
- If gels, blots, or fields are grouped or rearranged, then authors must insert spaces or dividing lines to indicate these changes and disclose the arrangement in the figure legend.

Mathematical Models

Presentation of the model(s) must be sufficiently clear to allow physiologists with limited experience in modeling to follow the model development, limitations, and physiological relevance. Assumptions concerning the importance of physiological processes included in the model should be clearly stated.

- If the model equation(s) require solution, the method of solution should be described in sufficient detail to permit readers to duplicate the solution in their own laboratories. Algorithms from commercial software libraries should be so identified. Details of the solution strategy may be summarized in an **Appendix**.
- For simulations, sources or estimation methods for all parameter values should be presented and the numerical values given in the text or a table. A sensitivity analysis must be performed for important parameters (covering ranges of values relevant to the manuscript) to determine how the model predictions are affected by numerical parameter values.
- If the model is used to estimate parameter values, measures of the uncertainties associated with the estimated parameter values should be presented.
- For models intended for use in a predictive setting, validation of the model with a data set not used for model parameter estimation (i.e., cross-validation) is recommended. Sensitivity analysis or parameter uncertainty determination is an important component of modern modeling practice that allows assessment of the validity of a model.
- Results obtained with the model(s) should be compared with appropriate physiological data, either from literature or from new experiments. Simulation results may be examined for prediction of changes or trends in physiological variables similar to those reported for in vitro or in vivo studies. The discussion should include information on the physiological significance of the model study, limitations of the model, and suggestions for new modeling and/or experimental studies.

Statistical Analyses

Provide the experimental data and results as well as the particular statistical significance of the data.

APS has published an **editorial** on the use of statistics, and authors are encouraged to consult it.

Data Repositories

Data reported in the manuscript should be stored in appropriate repositories such as those found at the National Center for Biotechnology Information (NCBI) and the European Bioinformatics Institute (EBI).and the repository used should be reported in the Methods Section. Data that should be deposited include:

- Nucleic acid/amino acid sequences (Genbank)
- Sequence data (UniProt)
- Other special types of data (e.g., genomes, bulk submissions) may be submitted to:
  - DDBJ - DNA Data Bank of Japan
  - EMBL - EMBL Nucleotide Sequence Database
  - GenBank - National Center for Biotechnology Information
- Microarray data, complete data set, should be submitted to one of these two databases prior to manuscripts submission: the NCBI Gene Expression Omnibus (GEO); or the EMBL-EBI ArrayExpress repository. Information for accessing the microarray data should be included in the article's Materials and Methods
- Genome-wide datasets using next generation sequencing (e.g. massively parallel sequencing) should be submitted to a compatible database such as the Gene Expression Omnibus (GEO) using the instructions and data formats supported by the database. Examples of this include RNA-Seq, ChIP-Seq or sequence modalities designed to examine epigenetic regulation. Authors should provide the set of login credentials (username and password) that will allow referees access the data set during review, if it is set up as a private resource.

Anexo C – NORMAS DA REVISTA *EUROPEAN JOURNAL OF APPLIED PHYSIOLOGY*

Instructions for Authors

TYPES OF PAPERS

The journal publishes Original Articles, Editorials, Invited Reviews, Letters to the Editor, and Replies.
Instructions for submitting Review Proposals, and for the preparation of Invited Reviews can be obtained directly from the Reviews Editors Nigel Taylor (ntaylor@uow.edu.au) and Michael I. Lindinger (mi.lindinger@gmail.com).
Authors of Letters to the Editor find specific guidelines on the link below.

- Instructions for Authors of Letters to the Editor (pdf, 321 kB)

Article Requirements

To facilitate rapid publication manuscripts should be prepared carefully in accordance with the following requirements.
The Conflict of interest is mandatory for all articles types.
Original articles and Invited Reviews should be composed as follows.

- Title page (numbered as page 1)
- Abstract (except for Editorials, Letters to the Editor and Replies)
- Introduction
- Keywords
- Abbreviations
- Methods (also Technique or Case histories)
- Results
- Discussion (comments and comparison with other published results)
- Conclusions
- Acknowledgements
- Conflict of interest
- References
- Tables
- Legends
- Illustrations

Special Issues

• Contributions that are part of a Special Issue must include the following footnote on the title page:
"This article is published as part of the Special Issue on [title of the Special Issue]"

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Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

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Please follow the hyperlink “Submit online” on the right and upload all of your manuscript files following the instructions given on the screen.

TITLE PAGE

Title Page

The title page should include:

- The name(s) of the author(s)
- A concise and informative title
- The affiliation(s) and address(es) of the author(s)
- The e-mail address, telephone and fax numbers of the corresponding author

Abstract

Please provide a structured abstract of 150 to 250 words which should be divided into the following sections:

- Purpose (stating the main purposes and research question)
- Methods
- Results
- Conclusions

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Specific remarks on Abstract

The sections should describe briefly and concisely the background and aim/hypothesis of the investigation, the most important methods, the major results and the conclusions drawn. Major results should be presented quantitatively where appropriate, and changes reported must be expected to be statistically significant (e.g. write "endurance time increased from a ± b to c ± d min" and not "endurance time increased (P ‹ 0.01)"). The conclusion should highlight the physiological significance of the study and not be a repetition of the results. The abstract should not contain any undefined abbreviations and references may not be cited.

TEXT

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX.

- LaTeX macro package (zip, 182 kB)

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.
Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

SPECIFIC REMARKS ON ABBREVIATIONS, RESULTS AND CONCLUSIONS

Abbreviations

While authors are encouraged to use abbreviations when appropriate, these should be used sparingly and should conform to the convention of the subject area, e.g. for cardiovascular physiology, thermo-physiology and respiratory physiology. Abbreviations should be defined at first mention in the text and supplied as a list, in alphabetical order, to be included after the keywords , e.g.

Abbreviations:	
ANOVA	Analysis of variance
EMG	Electromyography
MU	Motor unit
MVC	Maximal voluntary contraction
RMS	Root mean square
sEMG	Surface electromyography

If there are no abbreviations, state "None" in the Abbreviation list.

Result section

The text in the Results section should be written in a way so that it can be understood by physiologically interested scientists who are not specialists in the particular field of the investigation. Excessive usage of field-specific abbreviations should be avoided. The outcome of statistical analysis should be reported in the text only when it was used to test a specified hypothesis. Detailed descriptions of aspects such as subjects involved in the study, including statistical analyses, are better presented in tables. Authors are encouraged to include as figures original records obtained during experiments, and not simply group-mean responses; for instance, representative original records can often be used to illustrate complex differences between groups.

Conclusions section

Authors should provide concise conclusions to their work and are encouraged to put their results into a wider physiological context. The conclusions must not just be a repetition of the results.

SCIENTIFIC STYLE

Please always use internationally accepted signs and symbols for units (SI units).

REFERENCES

Citation

Cite references in the text by name and year in parentheses. Some examples:

- Negotiation research spans many disciplines (Thompson 1990).
- This result was later contradicted by Becker and Seligman (1996).

- This effect has been widely studied (Abbott 1991; Barakat et al. 1995a, b; Kelso and Smith 1998; Medvec et al. 1999, 2000).

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

Reference list entries should be alphabetized by the last names of the first author of each work. Order multi-author publications of the same first author alphabetically with respect to second, third, etc. author. Publications of exactly the same author(s) must be ordered chronologically.

- Journal article

  Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. doi: 10.1007/s00421-008-0955-8

  Ideally, the names of all authors should be provided, but the usage of "et al" in long author lists will also be accepted:

  Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

- Article by DOI

  Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. doi:10.1007/s001090000086

- Book

  South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document

  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

- Dissertation

  Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see

- ISSN LTWA

If you are unsure, please use the full journal title.

For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.

- EndNote style (zip, 2 kB)

TABLES

- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

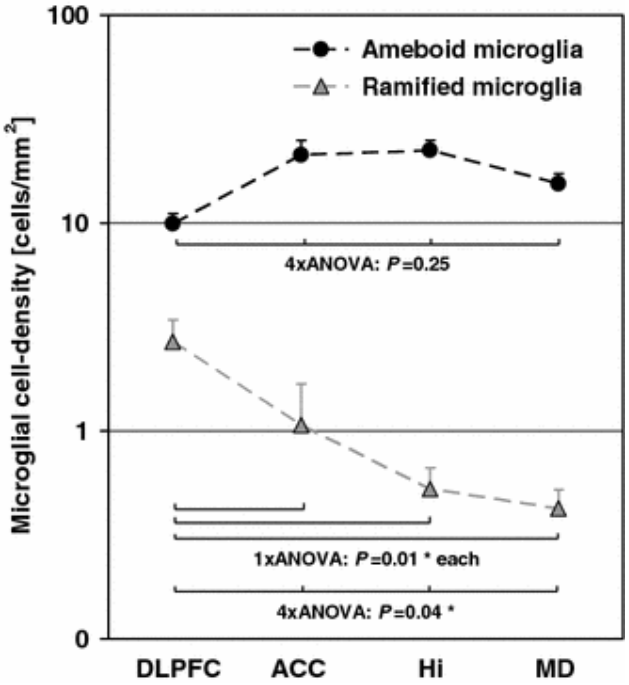
ARTWORK AND ILLUSTRATIONS GUIDELINES

Electronic Figure Submission

- Supply all figures electronically.

- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Line Art



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- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
- All lines should be at least 0.1 mm (0.3 pt) wide.
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
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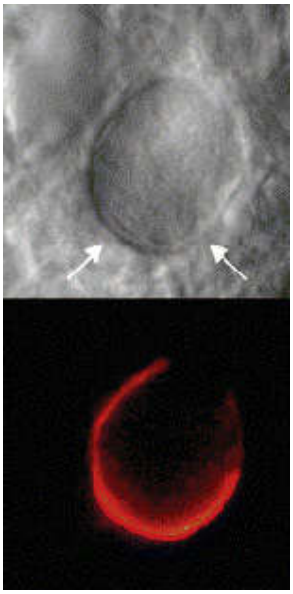
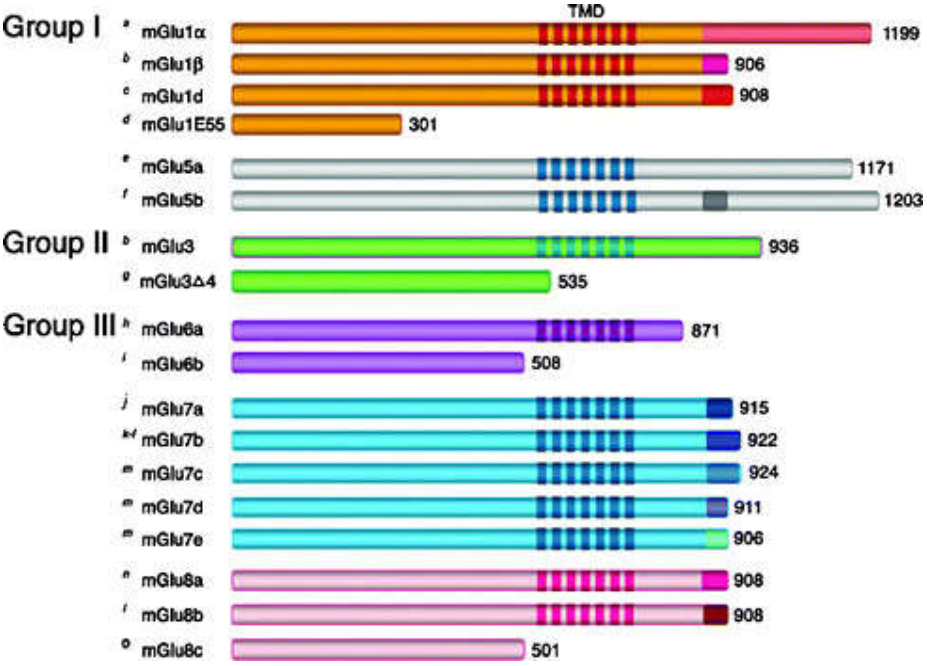
Halftone Art



- Definition: Photographs, drawings, or paintings with fine shading, etc.
- If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
- Halftones should have a minimum resolution of 300 dpi.

Combination Art



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- Combination artwork should have a minimum resolution of 600 dpi.

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- Do not include titles or captions within your illustrations.

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